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INVESTIGATION OF PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR RESEARCH IN SPACE BIOLOGY

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INVESTIGATION OF PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR RESEARCH IN SPACE BIOLOGY

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FOREWORD

This report presents the results of an eighteen month effort by Northrop Space Laboratories' Biologists to elucidate the basic biology and physiology of the pocket mouse. The work was supported by a contract between Northrop Space Laboratories and NASA (Contract No. NASr-91) following several years of preliminary studies supported wholly by Northrop Corporation.

A high level of interdisciplinary cooperation was required to fulfill the goals of this work. Physiologists, Geneticists, Radiobiologists and Ecologists worked together closely to reinforce each others studies and to make this report a meaningful composite. The full support and cooperation of managerial, technical and clerical personnel contributed materially to the successful completion of this task.

Many of the individual papers presented here were prepared for separate publication in scientific journals as well as for this report. It is hoped that the redundancy that might be noticed as a result of this dual purpose will not detract from the value of this report.

ABSTRACT

Pocket mice (Genus: Perognathus) are proposed as particularly suitable subject matter for space biology research by virtue of their unusual physiology. They have physiological characteristics which will permit significant simplification of the life support systems in biosatellites. The resultant savings in payload coupled with the small size of the animals permits the formulation of experimental designs involving statistically significant numbers of mammals, and a significantly higher probability of definitive results from this experimentation.

This report presents results of work in several broad biological areas. Specific data are presented on the following: oxygen requirements over a range of environmental temperatures for both normometabolic and hypometabolic animals; the cyclic nature of states of metabolic activity; response to whole body acute ionizing irradiation; blood values of normal and irradiated animals; and normal karyotypes of several species.

From these data are derived: (1) metabolic baselines delimiting the nature and complexity of a life support system for pocket mice in space vehicles; (2) baselines of selected physiological traits of pocket mice that are potentially useful for assessing the biological effects of extra-terrestrial residence; (3) specific design for space biology experiments utilizing pocket mice as test subjects.

TABLE OF CONTENTS

	Page
Foreword	i
Abstract	ii
Table of Contents	iii
List of Figures	iv-v
List of Tables	vi-vii
Introduction	1
Part I Metabolic Studies	2
1. Metabolic Characteristics of Pocket Mice (Perognathus) Especially the Little Pocket Mouse (P. longimembris)	3
Part II Radiobiology	60
2. Response of the Pocket Mouse to Ionizing Radiation	61
3. Metabolic Rates of Irradiated Perognathus longimembris	77
Part III General Biology	83
4. Blood Values of Pocket Mice (Perognathus)	84
5. Karyotype Analysis of Perognathus Species	95
6. Breeding of Heteromyid Rodents	102
Part IV Application	105
7. Applications to Space Biology Research	106
8. Life Support System for Biological Rhythms Experiment - A Preliminary Design	118

Detached for separate evaluation

LIST OF FIGURES

No. Page

Part I

Metabolic Characteristics of Pocket Mice

1. Schematic diagram of Metabolor 6
2. Photograph of Metabolor 7
3. Daily ranges of body temperatures of P. longimembris kept at constant ambient temperatures 13
4. Body temperatures of P. longimembris subjected to changing ambient temperatures 15
5. Relationship of highs and lows of metabolic rate and deep body temperature of P. longimembris 16
6. Regression of temperature gradient ($T_c - T_s$) on ambient temperature 18
7. Mean maintenance metabolic rates of Perognathus longimembris; measurements made with Metabolor 20
8. Maintenance metabolic rates of Perognathus longimembris; measurements with Beckman Paramagnetic Oxygen Analyzer 21
9. Maintenance metabolic rates of six species of Perognathus 27
10. Relationship between metabolic rate at 5°C and body weight for six species of Perognathus 28
11. Relationship between minimum maintenance metabolic rates at 35°C and body weight for six species of Perognathus 29
12. Maintenance metabolic rates of P. longimembris as measured in air and 80-90% oxygen atmosphere 34
13. Metabolic rates and body temperatures during entry into and arousal from periods of deep hypometabolism 36
14. Incidence of torpidity of P. longimembris kept at T_a = 10°C but allowed food 41
15. Metabolic activity of 2 representative P. longimembris maintained at 24°C with food 45
16. Metabolic activity of a single P. longimembris maintained at 22°C with no food 46
17. Comparison of rhythm of hypothermic periods in two groups of three P. longimembris studied under different environmental conditions 47
18. Metabolic activity of 6 P. longimembris monitored together, normal photoperiod at 10°C with no food 49

No.		Page
19.	Metabolic activity of 6 P. inornatus monitored together in dark chamber at 10°C with no food	50
20.	Relative metabolic activity of 3 P. longimembris in "isolated" and "non-isolated" chambers	51
21.	Metabolic activity in P. longimembris (♀) following 1400 r acute whole body Co^{60} irradiation (animal at 22°C with food)	52

Part II

Response to Ionizing Radiation

1.	Acute mortality of mice exposed to varying doses of ionizing radiation	67
2.	Early hematological changes in peripheral blood of Perognathus longimembris following total body irradiation	73

Metabolic Rates of Irradiated Mice

1.	Integrated oxygen consumption of 8 individually monitored Perognathus longimembris following exposure to 1400 r Co^{60} radiation	79
2.	Maintenance metabolic rate of normal normothermic Perognathus longimembris compared with that of irradiated P. longimembris	80

Part III

Karyotype Analysis

1.	Karyotype of P. flavus female, X3200	98
2.	Karyotypes of P. amplus female and P. longimembris male, X3200	99
3.	Karyotype of P. formosus male, X3200	100
4.	Karyotypes of P. baileyi female and P. fallax female, X3200	101
5.	Karyotype of P. penicillatus male, X3200	102

Part IV

Life Support System

1.	Life support system schematic	122
2.	Module location	123

LIST OF TABLES

No. Page

Part I

Metabolic Characteristics of Pocket Mice

1. Comparative data on minimum resting metabolic rates of species of desert-inhabiting rodents 23
2. Summary of data on six species of Perognathus 26
3. Maintenance metabolic rates of six species of Perognathus 26
4. Physical cooling characteristics of three species of Perognathus 32
5. Minimum rates of metabolism of P. longimembris in deep hypometabolic states 37
6. Metabolic "costs" and "savings" of P. longimembris during periods of hypometabolism 38
7. Frequency of torpidity in experimental group over 140 days of observation 40
8. Torpidity and survival of two groups of P. longimembris: never torpid versus frequently torpid 43
9. Summary of data on metabolic rhythms of Perognathus. P. longimembris is the species involved unless otherwise stated 54

Part II

Response to Ionizing Radiation

1. Experimental design 63
2. Blood values of 25 adult male Perognathus longimembris and 25 controls 68
3. Total blood cell counts of pocket mice 69
4. Per cent lymphocytes of pocket mice 70
5. Selected blood values of pocket mice 71

Part III

Blood Values

1. Peripheral blood values of adult Perognathus longimembris 88
2. Size of formed elements in Perognathus longimembris blood 90

No. Page

3. Hemoglobin and platelet values of female adult Perognathus longimembris 90
4. Peripheral blood values of adult Perognathus formosus 91
5. Heart blood versus tail blood of adult Perognathus longimembris 91

Karyotype Analysis

1. Comparative karyotype analysis of seven species of Perognathus 104

Part IV

Life Support System

1. Operational limitations 119
2. General metabolic requirements of the specimen 119
3. Earth metabolic requirements at S.T.P. conditions per specimen day (24-hours) 119
4. Integrated metabolic values for systems specified in tables 1 and 3 121
5. Total metabolic turn-over for complete experiment during specific mission 124
6. Components for specimen module 124
7. Components for experiment support module 125
8. Total weight, volume, and power equipment 126

INTRODUCTION

The inherent variability of biological material, and the nature of latent responses of organisms to chronic stresses suggest that the consequence of extra-terrestrial habitation will not be readily resolved through experiences involving human subjects. Therefore, irrespective of the progress of manned space flight, sophisticated research on lower organisms must be pursued in space, concurrent with manned missions, throughout the foreseeable future.

The cost per unit weight, coupled with the necessary complexity of packages containing living organisms, places a major limitation on the design of biological experiments to be conducted in space vehicles. Because of this limitation, it is not feasible at the present time to pursue programs which include sufficiently large numbers of mammals to insure statistical validity of the data obtained in single flights. Even when such experiments become possible through the development of less expensive methods, the inclusion of a large number of animals in any single flight is desirable in order to offset inherent biological variability and experimental error. This will lead to a greater flexibility in the design of experiments and a more efficient and rapid pursuit of research programs.

An obvious means of facilitating this development in space biology experimentation is to find a small mammal which demands a minimum of life support equipment and which is adaptable to the requirements of foreseeable research programs. Several species of pocket mice appear to fulfill these requirements.

Pocket mice are members of the genus Perognathus and, with the relatively rare Kangaroo mice (Microdipodops), form a major division of the rodent family Heteromyidae. Other members of this family are the Kangaroo Rats (Dipodomys), and the less familiar spiny pocket mice (Liomys and Heteromys). As far as is known, none of these animals require liquid water in their normal habitat or in the laboratory. Their requirements are met through the use of metabolic water, supplemented by the water in food consisting of air dry seeds.

Characteristics which suggest their potential use for space biological research are: small adult body size, a normal and complete absence of a requirement for intake of water, and a hibernation and estivation behavior pattern controlled by ambient temperature and available food supply. Their small size and weight will permit relatively large numbers of animals to be used in one biopack experiment. The absence of a water requirement greatly simplifies the design and weight of a life support system. Their unusual hibernation - estivation behavior suggests the possibility of maintaining the animals in a normal state of inactivity over periods of several weeks, or to induce or arouse them from torpor by changing the ambient temperature. In other experimental mammals it has been observed that the effects of irradiation remain latent in hibernating animals, and become manifest only after arousal to a normal state of activity. Post-hibernation laboratory observation of animals exposed in a dormant state to extra-terrestrial experiences would facilitate the study of biological responses to both space radiation and weightlessness.

The unusual traits of pocket mice do not disqualify them as representative mammals from which much experimental data applicable to man may be extrapolated. Unfortunately, however, insufficient information is available concerning the basic biology of these animals. One objective of the investigation reported herein was to obtain basic biological information as a step in the development of the pocket mouse into a unique research subject.

The genus Perognathus consists of a complex of species indigenous to the western United States and extending from Minnesota into northwestern Mexico. The species of interest in this investigation are found in the deserts of southern California and Nevada, and in Arizona. They differ in local habitat, behavior and adult size. Those studied as a part of this contract are listed:

Species	Distribution	Adult Body Weight
P. flavus	Arizona	6 grams
P. longimembris	Cal., Ariz., Nevada	6-10 grams
P. amplus	Arizona	15 grams
P. formosus	Cal., Nevada	15-22 grams
P. penicillatus	Cal., Ariz.	15-20 grams
P. intermedius	Arizona	15-20 grams
P. fallax	California	20-25 grams
P. baileyi	Cal., Ariz., Mexico	26-28 grams

Since body weight is a primary factor in selection of a species, P. longimembris and P. flavus appear to be the most promising. They are among the smallest of North American rodents, and are probably the smallest mammals potentially suited to the

purpose. P. longimembris received the most attention in this study because of its availability.

Small body size is, however, associated with a more critical temperature regulation mechanism because of the greater surface area to volume ratio. Since the control of activity is temperature dependent, some of the larger species may prove to have characteristics which would result in their selection in spite of their greater weight. Availability, breeding behavior, radiation sensitivity characteristics, or chromosomology of the larger species might also prove favorable to their selection.

The specific objectives of the research reported herein were to:

1. Determine those metabolic baselines that would define the nature and complexity of a life support system for pocket mice in space vehicles.

2. Investigate selected physiological traits present in pocket mice that are potentially useful for assessing the biological effects of extra-terrestrial residence.

3. Design specific space biology experiments.

METABOLIC CHARACTERISTICS OF POCKET MICE (PEROGNATHUS) ESPECIALLY THE LITTLE POCKET MOUSE (P. LONGIMEMBRIS)

R. M. Chew, R. G. Lindberg and P. Hayden

INTRODUCTION

The Little Pocket Mouse is particularly interesting with regard to its potential use for biological research in space: (1) Its small size (8-11 gm body weight) will allow a statistically significant number of animals to be placed in orbit. (2) Individuals are easily induced to hibernate, as shown previously (1). The low metabolic requirements of torpid animals permit a fairly long orbiting of a test group. The possible delayed effect of radiation in hibernating mammals may be useful in allowing one to ascertain the details of effects of prolonged space flight. (3) Since pocket mice do not need drinking water, the problem of their maintenance in flight is further simplified.

The purposes of the present study are to:

(1) determine the oxygen requirements of pocket mice in both normal and hypometabolic states

(2) to study the relationships of body temperatures to metabolic rates under different conditions

(3) to study physiology of pocket mice under conditions which induce hibernation, and ways of artificially inducing prolonged hibernation.

METHODS AND MATERIALS

Measurement of Oxygen Consumption:

Oxygen consumption has been measured in two ways: (1) With a closed-system multiple-channel Metabolor which can measure and record metabolic rates simultaneously from 9 animals. (2) With a Beckman G-2 Paramagnetic Oxygen Analyzer (P.O.A.) in an open system arrangement so that it continuously measures and records the oxygen

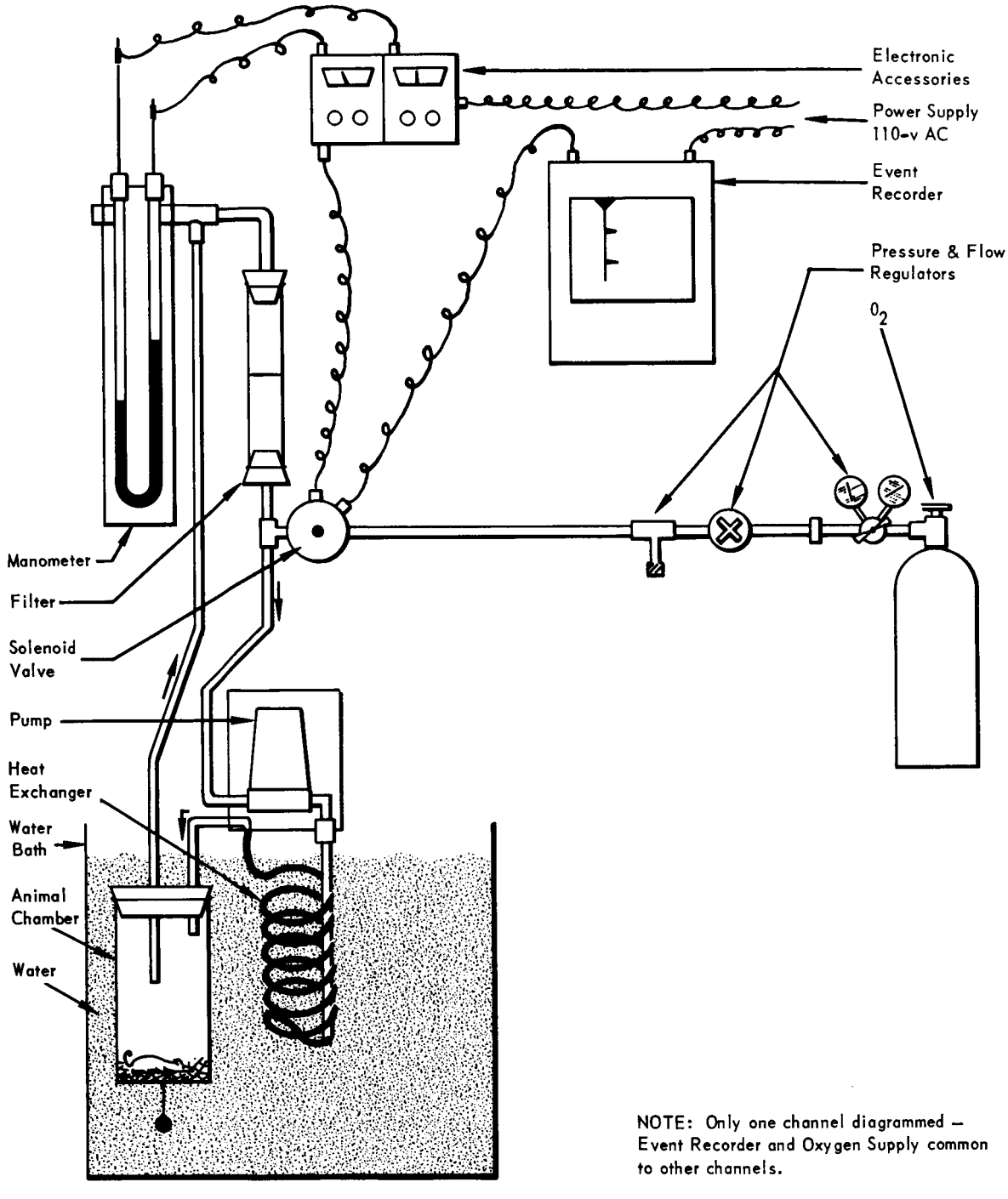

Figure 1. Schematic diagram of Metabolor.

Figure 2. Photograph of Metabolor.

concentration of an airstream passing through the animal chamber. This method can record from only one animal at a time, or from a group of animals collectively.

Multichannel Metabolor

Description - This instrument (Fig. 1,2) was specifically designed for use in long-term measurements of metabolic rates of normal and torpid pocket mice. The apparatus has nine independent units, permitting nine animals to be monitored individually and simultaneously.

The basic procedure of operation is that the experimental animal is (1) sealed in a closed system with circulating oxygen-containing atmosphere, (2) carbon dioxide and water vapor are absorbed continuously, (3) hence oxygen is the only component changed in the enclosed atmosphere, (4) as oxygen is consumed, pressure drops within the closed unit, (5) after a unit pressure drop, the oxygen is replenished.

The six major components of the device as shown in Figure 1 are: a constant temperature water bath; a unit which contains circulating pumps, connectors for animal chambers and heat exchangers; a wall panel with sensing manometers, solenoid valves, electronic accessories and gas absorbers; a 20-channel event recorder; an oxygen supply; and animal chambers. (See Fig. 2.)

The wide range constant temperature water bath (American Instrument Company Model 4-8605) has a range of -29° C to $+71^{\circ}$ C with a capacity of 50 gallons. Resting upon the water bath is a stainless steel unit which houses the circulating pumps (Thiberg, #1 aquarium pump), tubing from sensing manometers, and quick disconnect fasteners (Imperial fittings) leading to the animal chambers. Extending from the bottom of this component into the water bath are the heat exchangers (each composed of approximately 18 ft of 1/4-in. copper tubing).

The sensing manometers (Meriam Instrument Co. Model 20VD25) are mounted on a wall panel. These manometers are fitted with adjustable contacts within the manometer tube. When the manometer fluid touches these contacts the electronic circuitry external to the manometer (Meriam Manotac Model 70AA12) is opened or closed. The electronic circuit contains a thyratron tube which activates a 110-volt relay that controls a solenoid valve. The solenoid valves are normally closed but are opened periodically to admit an amount of pure breathing oxygen to the system. A 20-channel event recorder (Esterline-Angus Model A620T with Tempen) is connected to the solenoid circuitry and records when the solenoids are actuated.

A combination filter is provided on the wall panel to remove, respectively, water vapor and carbon dioxide from the air stream by the chemical action of Anhydron (R) (H_2O absorbent) and Ascarite * (CO_2 absorbent). Charcoal is added to the

*Registered Trade Name

filter mixture to remove noxious odors and gases that might occur in a closed system.

The oxygen supply is of the type employed in aircraft "breathing oxygen", 99.5% pure, and is supplied from a 220-cu ft bottle with a two-stage regulator which reduces the oxygen to 5-lb line pressure. The 5-lb line is reduced with a single-stage secondary regulator and variable flow restrictors so that the oxygen supply can be precisely controlled.

The animal chambers are 1000-ml tall-form spoutless beakers fitted with #15 rubber stoppers, bored for incurrent and excurrent air tubes. In some experiments 280-ml Mason jars have been used.

Operation - An animal is placed in the beaker with a constant amount of fine sand and either given food, or not, as required for the experiment. The beaker is sealed with the stopper, submerged completely in the water bath and air lines attached to the measuring system. Each of the nine systems is a self-contained, sealed unit with the pump acting only to circulate air through the absorbers and assure the animal of a sufficient supply of filtered atmosphere.

As the oxygen is utilized (carbon dioxide and water are continuously removed from the system), there is a decrease in the pressure within the system which causes the fluid in the right arm of the manometer to rise (the left arm is exposed to the atmosphere). When the fluid in the right arm of the manometer touches the sensing rod, the thyratron tube is fired, a relay is energized and locked, and a solenoid valve is opened which admits oxygen to the system. As pressure is increased the fluid in the left arm of the manometer rises; when it has risen far enough to touch the left sensing rod, the relay is de-energized and the solenoid valve closes, cutting off the oxygen supply. The system has now been recharged with the amount of oxygen utilized by the experimental animal. During the period when the solenoid valve was open, while the system was being recharged with oxygen, the pen of the event recorder was displaced from its normal position, causing a "pip" to appear on the permanent record.

The volume of oxygen replaced by the manometer-solenoid mechanism is calibrated for each unit in the Metabolor, at temperatures from 5° to 35°C, by withdrawing known quantities of gas from the system into a syringe maintained in the water bath at the same constant temperature as the animal chambers. The manometers were adjusted to give refill volumes of about 20-ml_{STP} at 25°C. Actual refill volume for the 1000-ml beaker animal chambers ranged from an average of 19.98-ml_{STP} at 5°C to 21.25-ml_{STP} at 35°C. Volumes for the 280-ml jars averaged about 10.50-ml_{STP} at 10°C.

From the volume of gas represented by the calibrated displacement of the manometer fluid and the times between "pips", milliliters of oxygen per unit time may be

calculated. All volume changes are corrected to STP. Since as many as nine animals can be run at a time, reasonable statistical analysis is always possible. Since sensitivity of the system is a function of the largest displacement of the manometer for the smallest removal of gas, sensitivity is improved by the use of the smaller 280-ml animal chambers. The Metabolor automatically records oxygen utilization over extended periods of time. Experimental periods of 120 hrs are common (with short periodic interruptions for gas absorber changes). The length of time that an animal can be left undisturbed in the experimental chambers is limited by the metabolic reserves of the animal or the amount of food supplied, or both. The device does not require experimental maintenance other than renewal of the gas absorbers. This device is capable of measuring very low utilization rates. Oxygen consumption rates of 0.040-ml gm/hr have been measured, and theoretically there are no rates too low to measure.

Atmosphere within the Metabolor Units - Since it is technically desirable to have a one-gas system in space biopacks, it was decided to use a high, nearly "pure" oxygen atmosphere at 760 mm Hg pressure in the present experiments. The actual oxygen concentrations within the operating Metabolor were checked with a Beckman Polarographic Oxygen Sensor.

Before an animal chamber was connected into the Metabolor, it was flushed for 30 seconds with oxygen; this was sufficient to bring it to 99.5 - 100% oxygen atmosphere. However, when the chamber was connected in line with the circulating pump, valves, filter and tubing, the residual air in these parts diluted the total circulating atmosphere to 90% oxygen (10% nitrogen). This concentration was then maintained during operation until it was necessary to change the filter. Each time the filter is changed, oxygen concentration within the total system is reduced 3%. With daily filter changes this would give a final oxygen concentration of 71% at the end of one week; with only two exceptions, the Metabolor was restored to 90% oxygen atmosphere at the end of a week of continuous operation. When pocket mice were hypometabolic, the filters were changed less frequently so that oxygen concentration varied from 90% down to 80% in a week's time. Most experiments involved the 90 to 80% oxygen concentration range. (In those experiments where it was desired to use normal oxygen concentration (21%) the animal chambers were simply flushed with air and connected into the circulating closed systems.)

The anhydrous magnesium perchlorate (Anhydrone(R)) in the filters kept the water vapor in the circulating atmosphere below 10% relative humidity, and possibly below 5%, as indicated by an American Instrument Company Electric Hygrometer (this instrument has limited sensitivity in the 0% to 10% relative humidity range).

No attempt was made to measure the concentration of carbon dioxide in the operating system, Ascarite (R) was assumed to remove all but a trace of the carbon dioxide released by the mice. Ascarite (R) is used in many chemical procedures for CO_2 absorption, with apparent effectiveness.

Effect of High Oxygen Atmosphere:

A series of tests were run to test whether the high oxygen atmosphere in the Metabolor had an effect upon metabolic rate of pocket mice. The results as reported in following sections strongly suggests that there is no effect of 80 - 90% O_2 on metabolic rates over the periods of exposure tested (up to 4 days). However, from other experimental runs, there is evidence of an effect on health and survival in long term exposure in the Metabolor, which may be partly or entirely due to the high oxygen concentration. This needs to be tested further.

Comparison of Measurement Methods:

The Metabolor has the advantage of being able to provide data on a "large" number of animals simultaneously under the same conditions, and measurements can be made conveniently over as long a period as desired. This method has the disadvantage that it cannot show short term changes in oxygen consumption - its limit is measurement of the time needed for a pocket mouse to consume 10 or 20-ml O_2; further, the method can be used only with a dry atmosphere.

With the Beckman Paramagnetic Oxygen Analyzer, the rate of oxygen consumption is continuously measured, so that all fluctuations are recorded. The humidity in the air stream flowing through the animal chamber can be regulated to any desired value. However, only one animal can be measured at a time.

The two methods are complementary and give comparable results.

Measurement of Body Temperatures:

Body temperatures were measured with very small-diameter thermistors contained in small - diameter polyethylene tubing. Thermistors were inserted 2 cm rectally (deep body temperature or core temperature, T_c) and also beneath the skin in the dorsal lumbar region (subcutaneous temperature, T_s). At the most, these two thermistors would be about 0.75 cm apart; together they provide data on the temperature gradient, $T_c - T_s$.

The leads of the thermistor were taped to the base of the tail and then extended vertically to an opening in the cover of the animal chamber. A light weight polyethylene collar was put on the animal to hinder it in getting at the leads and further, the bases of the leads were protected for 3 inches by a light weight spring through which the leads were threaded. This arrangement did restrict the animals movements but allowed them to feed adequately.

When it was desired, T_c and T_s could be recorded on a Yellow Springs recorder (YSI 47 Telethermometer and YSI 80 Recorder). Such records were made for normothemic mice, mice cooling to and warming from periods of torpor, and for mice killed and allowed to cool to ambient.

Animal Sources and Housing:

Wild Perognathus were collected in the field from several locations in California: Whitewater Canyon, Deep Springs Valley, Barstow, Pearblossom, and also from Lathrop Wells, Nevada.

The species collected and studied include: P. longimembris, P. flavus, P. amplus, P. intermedius, P. formosus, P. baileyi, and P. inornatus.

Pocket mice were housed individually in wide-mouth gallon jars containing about 5 cm of sand. A mixture of parakeet seed, rolled oats, and sunflower seed was provided ad libitum. No drinking water was given but small bits of vegetable greens were fed occasionally. Mice were housed in rooms kept between 20 and 24°C, with relative himidity 45 - 55%. Photoperiod was controlled to 0600 to 1800.

One group of 50 - 70 P. longimembris were kept under the same conditions, except at 10°C and ~ 70 % relative humidity in a constant temperature room. A few animals were also kept at this temperature in 10 gallon aquaria with artificial "burrow systems" (described in detail later).

RESULTS AND DISCUSSION

Metabolism and Body Temperature of P. longimembris:

Body Temperature of Normometabolic Animals

Animals kept at a constant ambient temperature - As shown in figure 3, the daily range of T_c is considerable: at T_a of 5° it is 3.1-4.8° C; at T_a 10°, 2.6-4.5; T_a 15° 2.7-3.6; T_a 25° 1.1-4.3; and T_a 35° 1.9-2.5.

In animal No. B3♀ kept for 17 hours at T_a 10°, the extreme range of core temperature was 3.6°C (range 34.2 - 37.8). During individual hourly periods, body temperature varied an average of 1.51° (range 0.8-2.2°C). This range of core temperature is not surprising for such a small mammal weighing only about 10 g. Because of its small mass and high metabolic rate per unit mass, P. longimembris tends to warm up rapidly. Because of its large surface area per unit mass, it cools rapidly. Since there is only a small total amount of water in the body to buffer temperature changes, this small animal inherently has less temperature stability than a larger mammal.

The data of figure 3 suggest that the general level of body temperature is the same over the range of ambient temperatures 5 to 35° C. However, there are too few

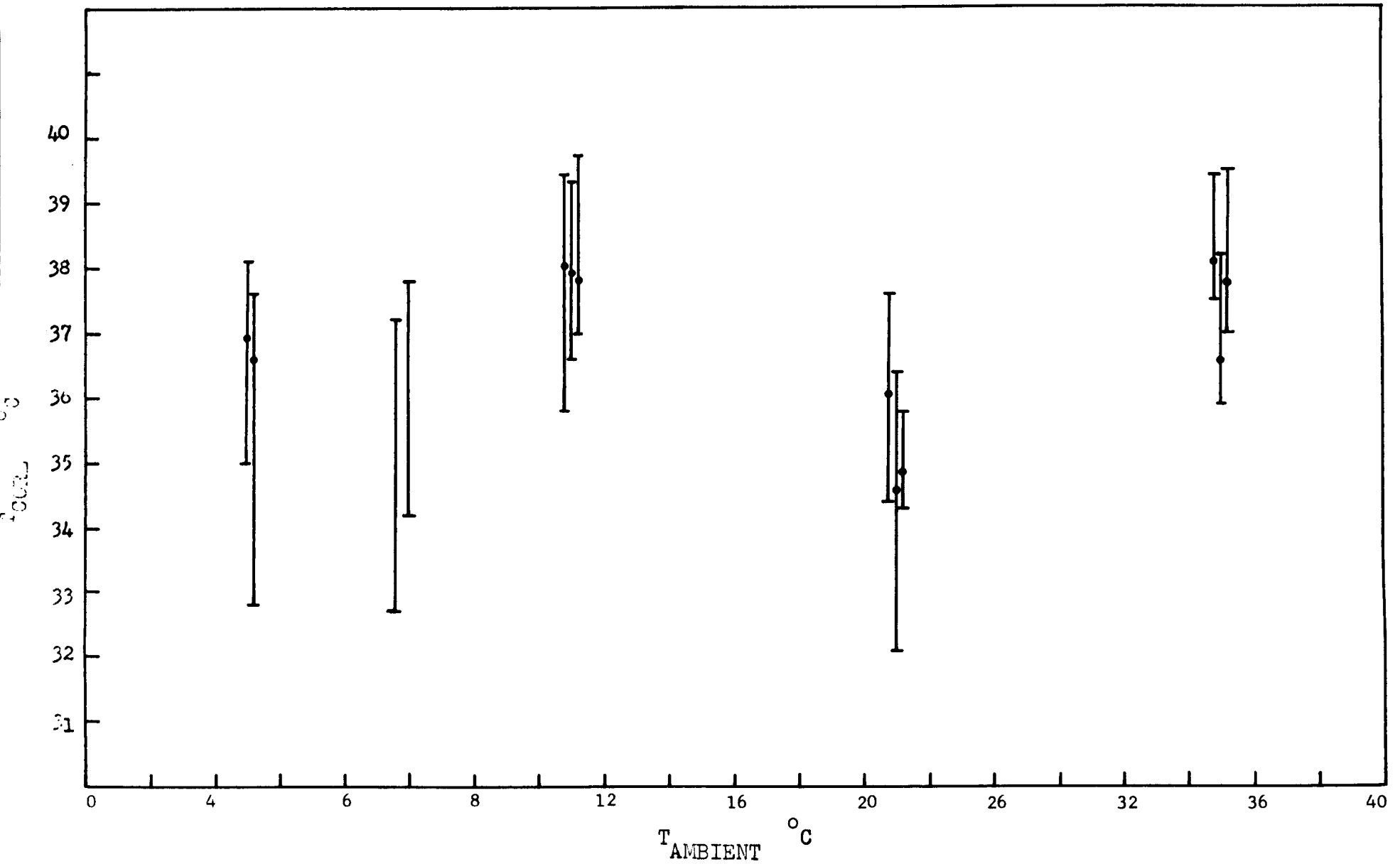

Figure 3. Daily ranges of body temperatures of P. longimembris kept at constant ambient temperatures. (Points represent mean body temperature for single animals; verticle line shows extreme range. Means not available on animals at 10 °C.)

data to permit drawing a firm conclusion.

Animals subjected to changing ambient temperatures - Core and subcutaneous temperatures were recorded on six P. longimembris while they were subjected to a changing ambient temperature; after an hour at room temperature, T_a was either lowered gradually to 2°C or raised to 38.5°C.

As shown in figure 4, with one exception, the mice were able to maintain a stable level of T_c over a range of ambient temperatures from 2° to 34°C, all animals showed a steep rise of core temperature at ambient temperatures above 34°C. P. longimembris are able to withstand, at least briefly, deep body temperatures of 41.5 to 42°C. Gross salivation was observed in the animal that reached a T_c of 42.0°. One animal survived a rise of T_c to 41.8°C, and then a second rise to 41.3°C, 1.75 hours later, after having been allowed to recover to normal range. This tolerance of high body temperatures, even if only briefly, may be of importance to pocket mice in nature, under exceptional conditions when they might be exposed to daytime surface temperatures in the desert.

Relationship between metabolic rate and core temperature - Because of the rapid heating and cooling rates of the body, body temperature can be expected to follow closely any changes to metabolic rate. This is shown in the data for animals which were held 24 - 48 hours at cold ambient temperatures.

For example, the data for animal B3♀, over a period of 17 hours at T_a of 10°C:

(1) Within each hourly interval the highest body temperature clearly coincided with the time of maximum oxygen consumption in 13 out of 17 (77%) hours. The lowest body temperature coincided clearly with minimum oxygen consumption in 10 of 17 (59%) of the hourly intervals (See figure 5).

The minimum body temperatures lagged behind metabolic lows by about 1.9 minutes, while maximum body temperatures lagged metabolic peaks by about 0.7 minutes (See figure 5).

(2) Within each hour, the 10-minute period with the lowest mean body temperature coincided with the 10-minute period with lowest metabolic rate in 12 of 17 hours (70%). The 10-minute period with highest mean body temperature coincided clearly with maximum metabolic rate only 4 out of 17 times (24%); however, it was often difficult to estimate just which of several 10-minute periods had the highest metabolic rate, as there were often several almost equal periods.

(3) The ranking of mean hourly body temperatures coincided with that of mean hourly metabolic rates:

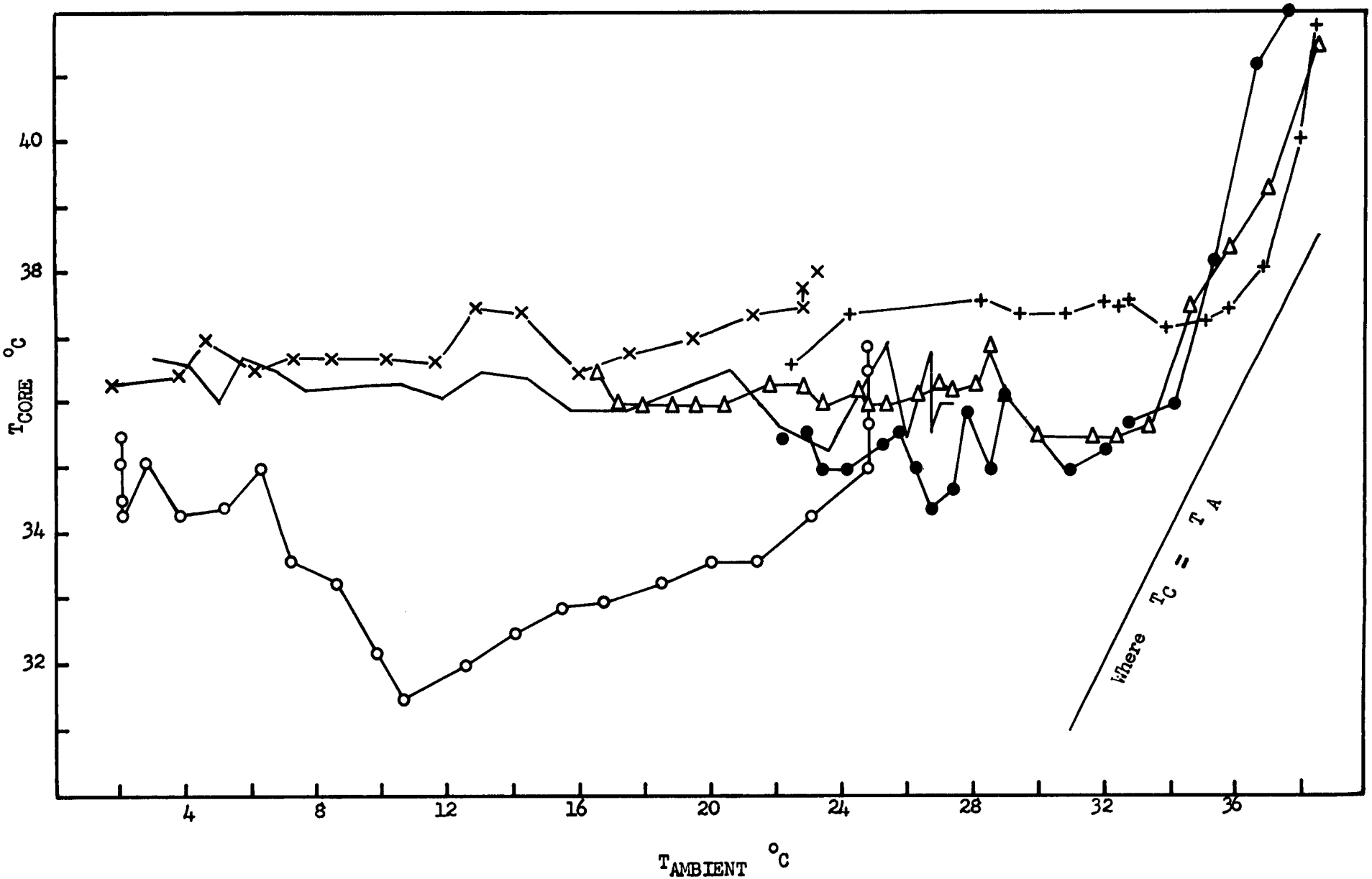

Figure 4. Body temperatures of P. longimembris subjected to changing ambient temperatures.

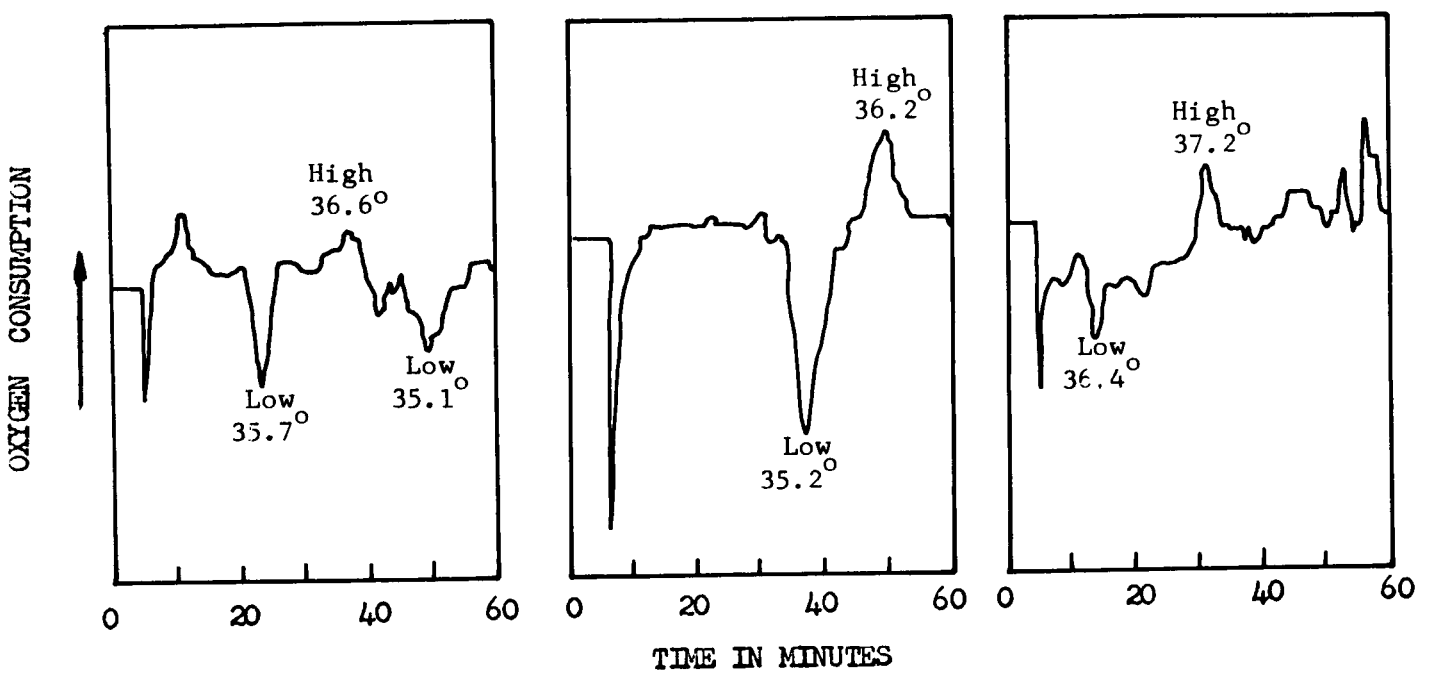

Figure 5. Relationship of highs and lows of metabolic rate and deep body temperature of P. longimembris.

Metabolic rate	Number of hours at this rate	Mean body temperature these hours
74.79 ml/hr	1	37.05°C
72.92	2	36.57
71.05	5	36.41
69.18	4	35.86
67.31	2	35.78
63.57	1	35.59

Relationship of the temperature gradient, $T_c - T_s$ to ambient temperatures - Figure 6 shows the regression of $T_c - T_s$ on ambient temperature for the data from 6 animals exposed to slowly changing ambient temperatures. The graph has three components: (1) Below a T_a of about 14°, down to at least 2°C, the gradient is approximately constant at 3.3°. The slope of the regression line is not significantly different from zero. (2) From T_a 28° to 38° the gradient is constant at about 0.97°C. (3) Between 14° and 28°, the gradient linearly increases with decreasing ambient temperature.

The data of figure 6, together with those of figure 4, suggest that there may be four zones of temperature regulation for P. longimembris:

(1) From T_a of 35° to 38.5° or higher. Figures 7 and 8 indicate that the metabolic rate reaches a minimum at an ambient temperature of 34-36°. Above this point, the constant heating resulting from the minimum metabolic rate, combined with a minimum body insulation, keeps deep body temperature at least 1.5° above environmental temperature. Equilibrium of heat input with heat loss occurs only when the body core is 1.5 to 4.0° higher than T_a, at which point T_s is about 1° less than T_c. In this zone of "regulation", as T_a increases, T_c increases in parallel.

(2) T_a 35° down to 28°. As T_a decreases below 35°, metabolic rate increases linearly (See figures 7 and 8). The fact that the gradient of $T_c - T_s$ remains constant suggests that within this range there is a progressive increase of insulation of the body, possibly due simply to erection of the fur, with maximum insulation being achieved at about 28°.

(3) T_a 28° to 15°. The linear increase in temperature gradient, although core temperature remains essentially constant, suggests that insulation remains constant at the maximum value achieved at 28°. Each increment of metabolism per unit drop of T_a maintains a constant core temperature, but allows the core-to-

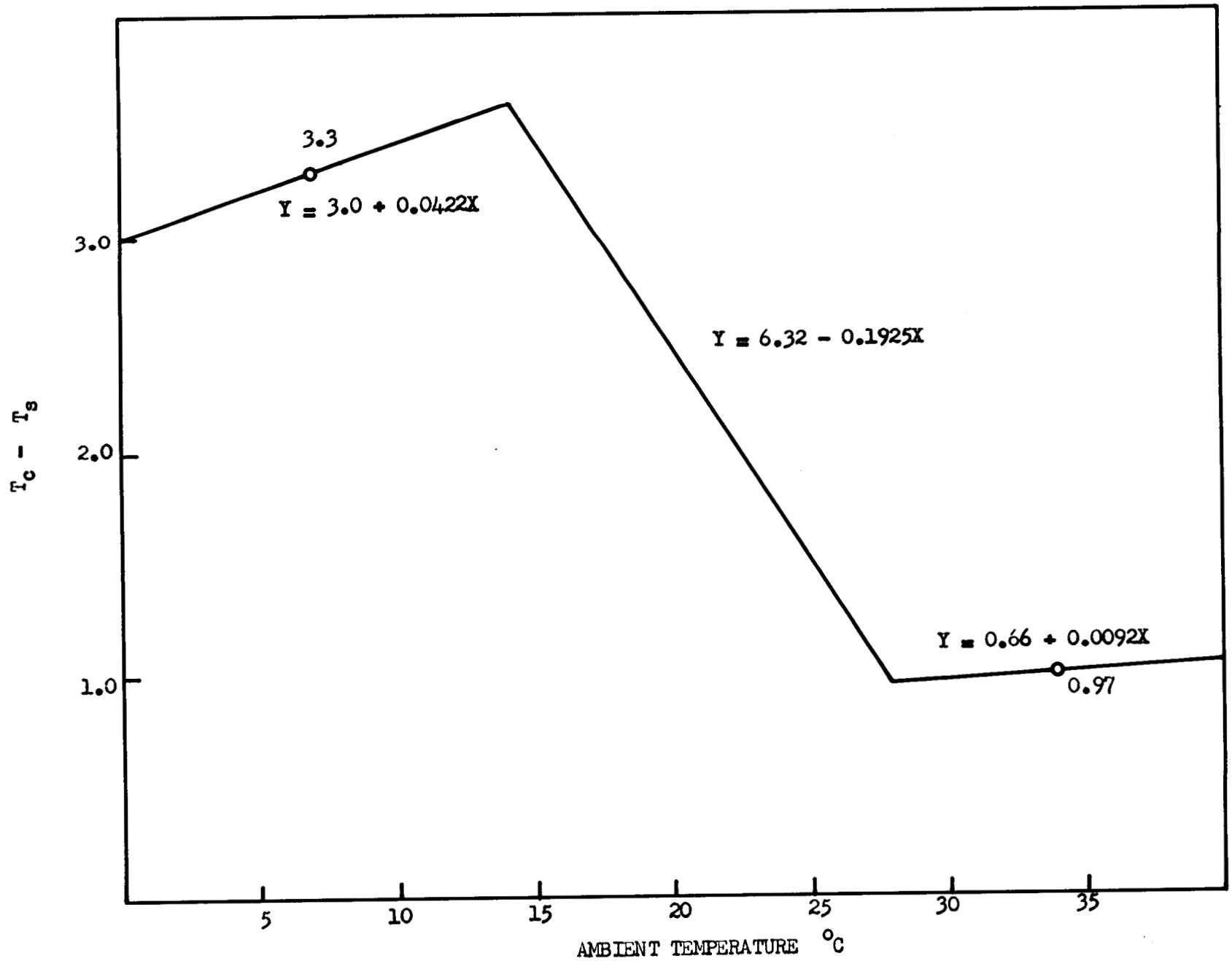

Figure 6. Regression of temperature gradient (T_C - T_S) on ambient temperature.

surface gradient to increase gradually.

(4) T_a below 15°, to at least $2^{\circ}C$. Although metabolic rate continues to increase at the same rate, as between 15° and $35^{\circ}C$, the gradient is stabilized at about 3.3°. This suggests that an additional factor of temperature regulation has been involved, i.e., some altering of the physical characteristics of superficial tissues so as to reduce their conductance. This could be a vascular adjustment, or possibly only a postural change which reduces the amount of exposed surface.

Metabolic Rate of Normometabolic P. longimembris

Mean maintenance metabolic rates - Pocket mice were placed in 1000-ml chambers with food and their metabolic rates were measured for 12 to 24 hours (usually 24) at a series of ambient temperatures (usually 4 different) over the range of 5° to $35^{\circ}C$. The average values from such measurements, ml O_2 STP/gm hr, are measures of the metabolic requirements of mice when simply maintaining themselves with limited activity in a confined space. Such values probably approximate those of animals remaining within their burrows in nature, and they are the basis for estimating the requirements of animals to be confined within small spaces in a biopack experiment.

The results from 6 experiments involving 53 different P. longimembris are graphed in figure 7. There are some significant differences between groups of animals, especially at lower temperatures. These differences may be attributed to seasonal changes in pelage and physiological characteristics, since the measurements are made over a 5-month span, to possible genetic differences among the wild populations from which the mice were taken, to somewhat different mean body weights, and simply to variation between individuals. The two groups of animals without food were not significantly different from the others which were provided seed ad libitum.

All the data are summarized in the regression fitted to the means of the data:

$$\text{ml } O_{2\ STP}/\text{gm hr} = 12.163 - 0.276\ T_a(^{\circ}C).$$

Thus a 9-gram Little Pocket Mouse will require an average of 109.5 ml O_2 per hour at $0^{\circ}C$ and a minimum of 22.53 ml per hour at $35^{\circ}C$.

Figure 8 gives the limited data, for three mice, obtained with the Beckman P.O.A. The slope of the line for these data is almost the same as the data of figure 7, and it is probable that the absolute levels of the lines are not significantly different.

Minimum maintenance metabolic rates - Values reported in the literature on metabolism of small rodents are usually in terms of the minimum resting oxygen consumption during the total measurement period. For purposes of comparison, minimum oxygen consumptions have been calculated for three sets of data:

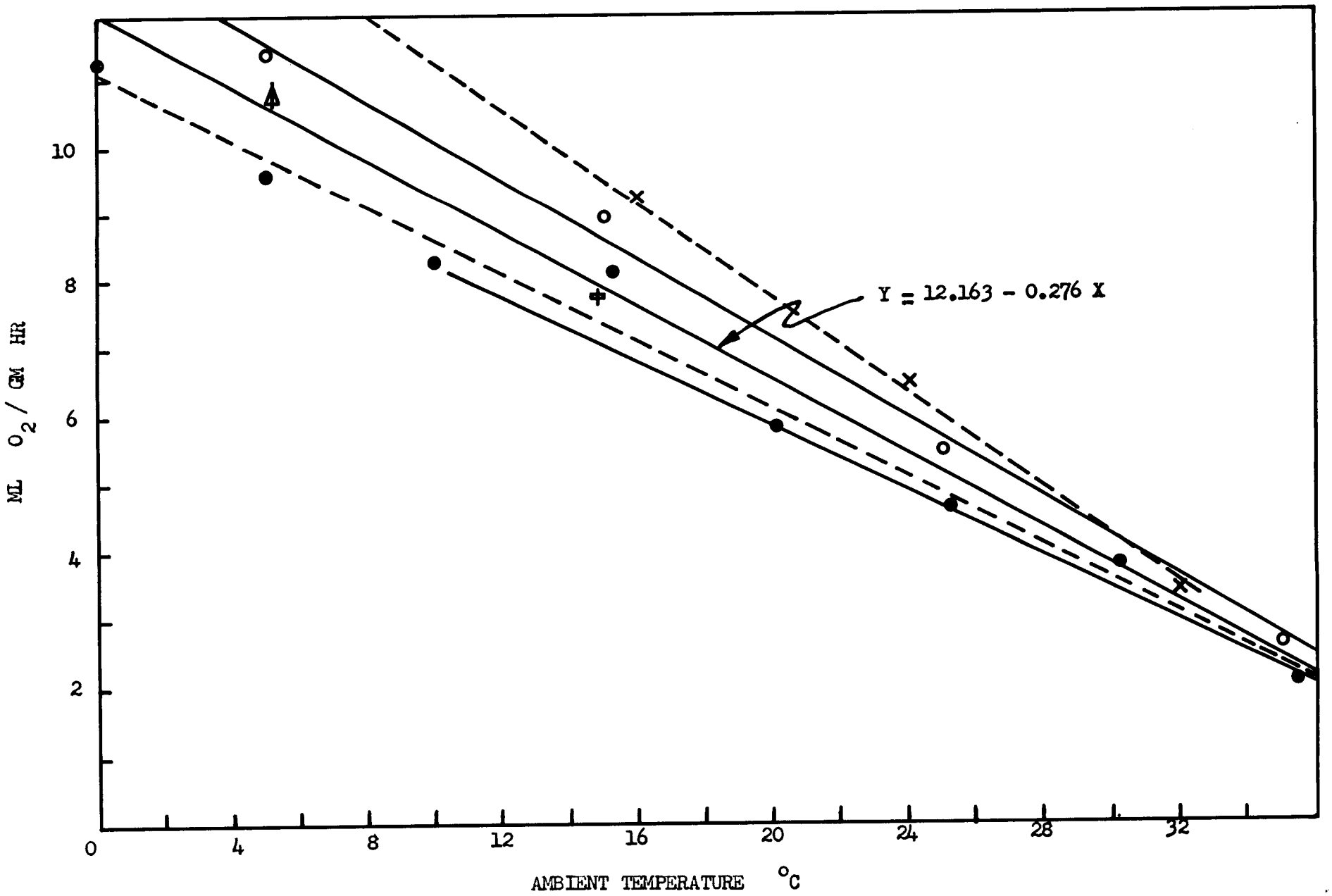

Figure 7. Mean maintenance metabolic rates of P. longimembris; measurements with Metabolor.

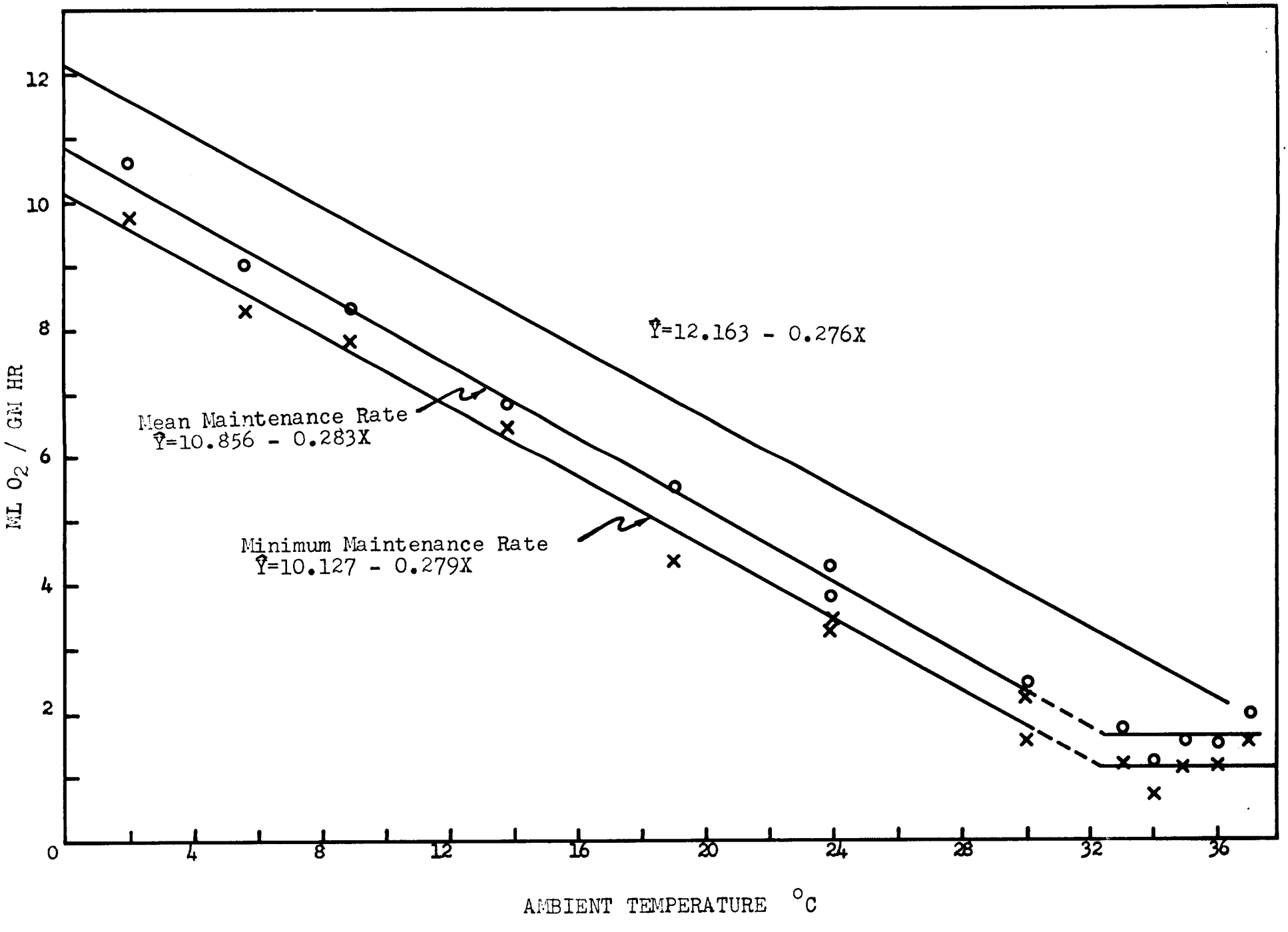

Figure 8. Mean maintenance metabolic rates of P. longimembris; measurements with Beckman Paramagnetic Oxygen Analyzer.

Source of data	Average maintenance metabolic rate	Minimum maintenance metabolic rate	Min. / Ave.	
			At 0°	At 30°
Data of figure 8[1]	Y = 10.856-0.283X	Y = 10.127-0.279X	93.3%	74.3%
Lathrop Wells[2] animals Fig. 7	Y = 10.68-0.239X	Y = 8.36-0.189X	78.2%	78.9%
Pearblossom[2] animals Fig. 7	Y = 12.221-0.266X	Y = 7.78-0.169X	63.7%	63.7%

(1) value for lowest 1/2 hour during continuous record of Beckman P.O.A.

(2) minimum values for utilization of one unit ($\sim$ 20 ml) in the Metabolor

The minimum rate is about 72% of average at T_a = 30°C.

Basal metabolic rate - P. longimembris has only a narrow zone of thermal neutrality, from about 33° to 36°C, as well as can be judged from figure 8. Metabolic rates increase above 36°C and the mice die at ambient temperatures of 38° to 40°C. The minimum metabolic rates for ambient temperatures within the zone to thermoneutrality approximate the basal metabolism of this species as nearly as such a value can be obtained. The animals were not fasting and may have been slightly hyperthermic at T_a 33-35°C, both of which conditions violate the technical definition of the basal state. Following are some representative "basal" rates:

Source of data	T_a	"Basal" metabolic rate
Figure 8 (animals in air)	33-36°C	1.130 ml O_2 STP/gm hr
Pearblossom animals Fig. 7 (90% O_2)	35°C	1.849 $\pm$ 0.302*
Lathrop Wells animals Fig. 7 (90% O_2)	35°C	1.759 $\pm$ 0.188*

*one standard deviation

Two sets of data were obtained for normothermic animals in a fasting state (Whitewater and Barstow groups, figure 7) at 5° and 15°C. Both are very close to the regression line for all means, and hence not significantly different from non-fasting mice at these temperatures. However, the same may not be true for ambient temperatures in the zone of thermal neutrality.

Discussion - There are few data with which the present information on the Little Pocket Mouse can be validly compared. The data of figure 7 are almost identical to maintenance metabolic rates of 9-gm harvest mice (Reithrodontomys megalotis) as measured by Pearson (18). Perognathus longimembris has a somewhat lower minimum value, and its zone of thermal neutrality is at a slightly higher temperature.

A 9-gm pocket mouse has a lower 24-hr metabolic rate at 27°C than a 9-gm meadow

vole (Microtus pennsylvanicus) as measured by Wiegert (25) P. longimembris 4.50 ml/gm/hr, vs M. pennsylvanicus 7.18 ml/gm/hr. Voles in the 9-gm range are only young animals; however, Wiegert found a constant rate per gram in the weight range of animals he studied, 11 - 50 gm.

Pearson (16) measured the 24-hr metabolism of a variety of small rodents, at 22 - 26°C ambient temperature. Average weights of different species ranged from 17 to 31 gm, and metabolic rates from 3.0 to 4.3 ml/gm/hr. The Little Pocket Mouse has a higher metabolic rate, on a per gram basis, than any of these heavier rodents. This is to be expected, since rate per gram tends to vary inversely with total body weight in mammals (4). The only mammal studied by Pearson that is in the pocket mouse weight range was the Big Brown Bat, Eptesicus fuscus. This species, 11.6 gm average weight, averaged 1.5 ml/gm/hr at 22 - 26°C. However, the bats were probably hypothermic for part of the 24-hr measurement time, and cannot be compared with normothermic pocket mice (Figure 7).

Data are available for several species of desert-inhabiting rodents; these are summarized in Table 1; however, all of these species are considerably heavier than the small pocket mouse.

Table 1. Comparative Data on Minimum Resting Metabolic Rates of Species of Desert-Inhabiting Rodents

Source (Ref)	Species	Body wt. (gm)	Metabolism-temperature relationship Y = (ml/gm/hr) X = T_a in °C	Basal rate (ml/gm/hr)	Thermal neutrality (T_a in °C)
2	Microdipodops pallidus	15.2	Y = 4.8-0.10X	1.3	35
6	Dipodomys merriami	34.7	Y = 7.11-0.10X	1.2	31-35
6	D.panamintinus	56.9	Y = 6.57-0.158X	1.2	33-34
6	Citellus leucurus	79.2	Y = 7.60-0.202X	1.3	31-34
20	Meriones pyramidum	72-145	Y = 3.88-0.102X	0.6	30
21	M.unguiculatus	61-80	Y = 5.66-0.141X	1.4	30-40
24	Perognathus californicus	20.9		0.97	32.5
Present Data	P.longimembris	8-10	Y = 10.68-0.239X	1.0	33-35

The Little Pocket Mouse has a basal metabolism in the same range as the other species, but its metabolism increases much more with decreasing ambient temperature. This is to be expected because of the much smaller size of P. longimembris; heat is lost much more rapidly (per unit weight) from a small body than from a large body. However, the data for the Little Pocket Mouse contrasts markedly with those for the kangaroo mouse, Microdipodops pallidus, which is a not much larger member of the same family of rodents (Heteromyidae). The kangaroo mouse is also easily induced to become torpid as cited by Bartholomew and MacMillen (2). The two species of gerbils, Meriones spp. are surprisingly different in their metabolism.

With regard to the logistics of maintaining normothermic pocket mice, it would be best to keep them as near their point of thermal neutrality (33 - 35°C ambient temperature) as possible, without danger of exceeding their upper lethal temperature. (38 - 40°C ambient). An ambient temperature of 30°C may be most suitable.

All of the desert rodents of Table 1 have basal metabolic rates below those predicted by the general "mouse to elephant curve" of Brody (4):

$$\text{kcal/day} = 70.5\text{kg}^{0.734}$$

For a 9-gm mouse, this formula predicts a metabolism of 2.22 kcal/day, or 2.1 ml (oxygen)/gm/hr (assuming an R.Q. of 0.82). The basal metabolisms of these rodents are much lower than the values predicted by the formula of Kayser (12), for basal metabolism of species that hibernate:

$$\text{kcal/24 hr} = 63.6\text{kg}^{0.62}$$

This formula predicts a rate of 3.43 kcal/day, or 3.29 ml/gm/hr, for a 9-gm mouse; therefore, there is a definite advantage to be gained in using a member of this group in experiments where the supply of oxygen is a critical logistics problem.

Maintenance Metabolism of Other Species of Perognathus:

Procedure - The maintenance metabolic rates of six species of Perognathus were measured in the Metabolor. Metabolic rates were measured for 24-hour periods at 5°, 15°, 25°, and 35°C for all species, and also at 30°C for the two larger ones. It was found that the best procedure was to alternate two groups, allowing the mice alternate "days off" at room temperature (20 - 24°C) between metabolic measurements. This alternation reduced or eliminated any loss of body weight during the temperature sequence. Mice were kept in 1000-ml beakers containing a layer of sand, and were given an excess supply of sunflower seeds.

The species studied are listed in Table 2, with body weight data. The species ranged from average weights of 7.54 to 30.95 grams. All of these species inhabit desert environments in the southwestern United States.

Results - Results are summarized in Tables 2 and 3, and in figure 9. In figure 9, maintenance metabolic rates are given in the form of linear regression equations of the general form, $Y = a + bX$, where Y is the metabolic rate (ml/g hr), the variable dependent upon X, ambient temperature (^{o}C); the value a is the metabolic rate at $0^{o}C$, and b is the slope of the regression line. The regression lines are calculated from the data for individual mice at $5^{o}C$, $15^{o}C$ and $25^{o}C$, all of which temperatures are below the critical temperatures for these species. The regression line for each species is extended to intersect a horizontal line drawn through the average metabolic rate at $35^{o}C$, which is within, or slightly above, the zone of thermal neutrality of these species.

The six species give a family of curves (Figure 9) in which there is a progressive increase in the value of a and the slope of the curve (value b) as one proceeds from the heaviest to the lightest species.

If the regression lines are projected to base line (where $Y = 0$), they intersect at points within the range of normal body temperatures for pocket mice, except in the case of P. longimembris, which intersects at the base line at $45.5^{o}C$.

In figure 10, maintenance metabolic rate at $5^{o}C$ is plotted versus body weight, for individual mice. The 24-hour data were analyzed in four 6-hour units, and in figure 11 are plotted the values for the 6-hour period with the lowest average metabolic rate, at $35^{o}C$, versus body weight. These values at $35^{o}C$ approximate minimum resting metabolism or "basal" metabolism of these species.

Discussion -

(1) Metabolic rate in relationship to ambient temperatures - A family of metabolism curves, as in figure 9, is to be expected on the basis of the well-known inverse relationship between metabolic rate (expressed per gram of body weight) and total body weight (see Brody, 1945, for example). Conversion of the regression lines within the range of normal body temperatures, (when $Y = 0$), indicates that these mammals, like most others, lose heat in a simple and direct fashion, following Newton's law of cooling (23, 15).

Even with furs of the same insulative value, a small mammal will lose heat more rapdily than a larger species, because of its greater surface area exposure per unit of mass. The slope of the regression line is a measure of the integrated heat conductance of the mammals' bodies, integrating both physical and physiological factors that influence heat loss. For the present data, conductance values range from 0.40 ml O_2/g hr ^{o}C for the smallest species, P. flavus, to 0.18 ml O_2/g hr ^{o}C for the largest, P. baileyi. The largest conductance noted in the literature, for a mammal in an environment below its critical temperature is 0.6 ml O_2/g hr ^{o}C, recorded by

Table 2. Summary of Data on Six Species of Perognathus

Species	Average wt. (grams)	Relationship of Maintenance Metabolic Rate (Y) to Ambient Temperature (X) in Range of 0°C to 30°C	Theoretical X when Y = 0 (degrees °C)
flavus	7.54 ± 1.18	Y = 15.007 - 0.405X	37.1
longimembris	8.22 ± 0.70	Y = 12.249 - 0.269X	45.5
amplus	12.84 ± 1.57	Y = 10.982 - 0.283X	38.8
intermedius	12.96 ± 1.80	Y = 9.669 - 0.242X	39.9
formosus	18.29 ± 1.46	Y = 8.324 - 0.216X	38.5
Baileyi	30.59 ± 5.08	Y = 7.272 - 0.185X	39.3

Table 3. Data Maintenance Metabolic Rates of Six Species of Perognathus

Species	Maintenance Metabolic Rate Over 23-hr Period (ml/g hr)				
	5°C	15°C	25°C	30°C	35°C
flavus	13.129	8.646	5.036		2.417
longimembris	10.853	8.334	5.473		2.941
amplus	9.684	6.506	4.025		1.815
intermedius	8.675	5.841	3.707		1.944
formosus	7.180	5.213	2.856	2.260	2.029
Baileyi	6.300	4.584	2.605	1.914	1.826

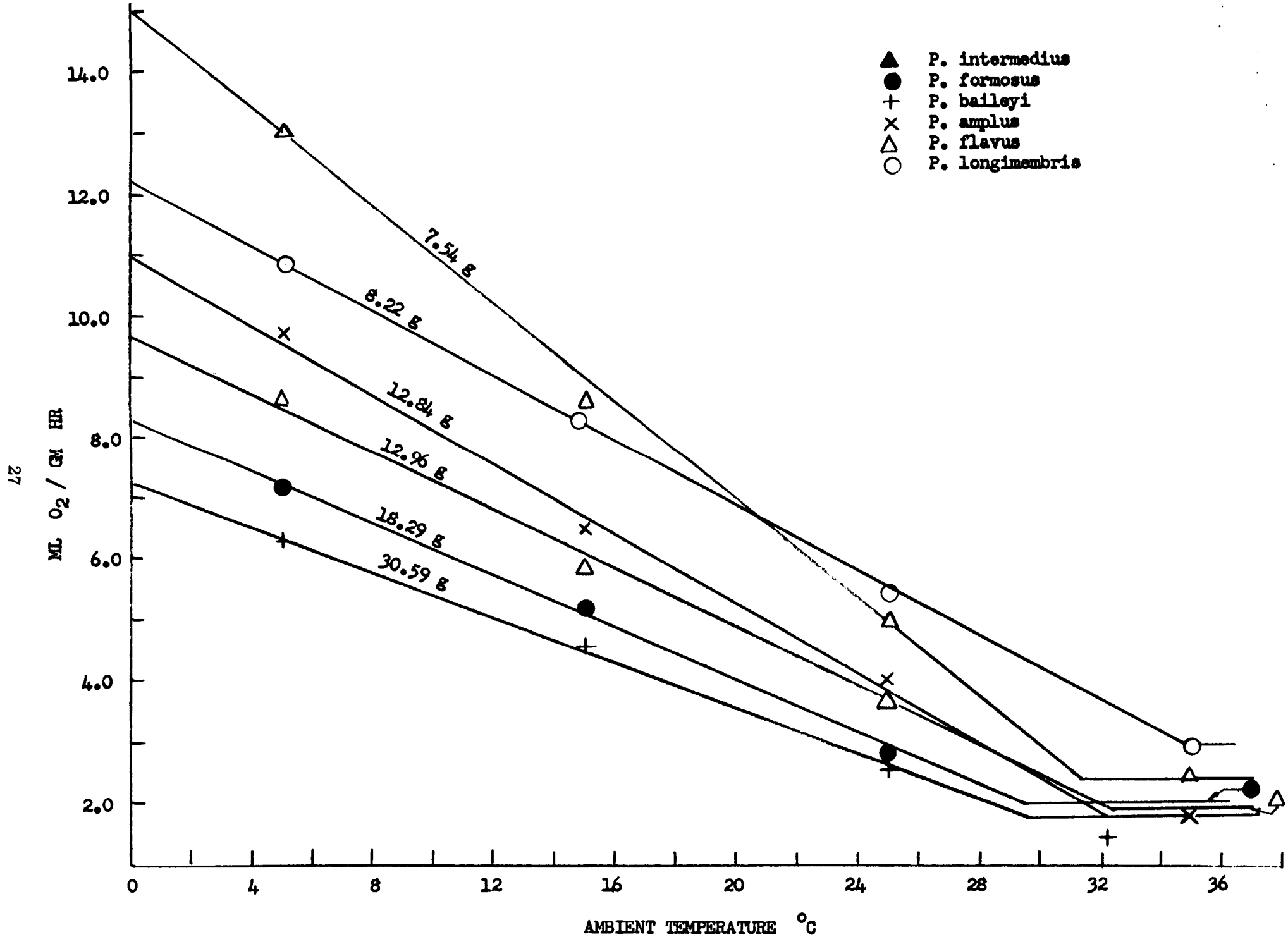

Figure 9. Maintenance metabolic rates of six species of Perognathus.

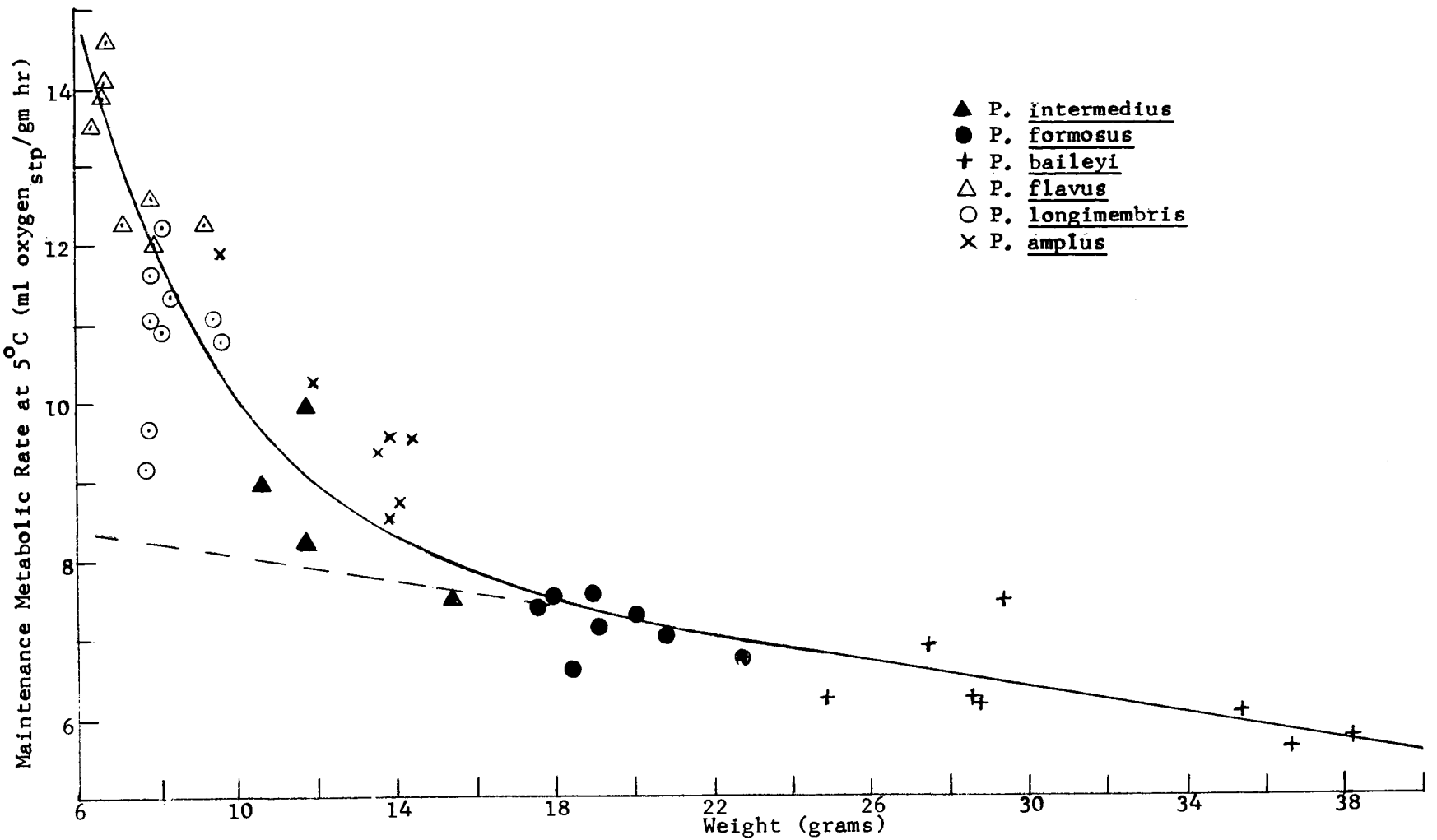

Figure 10. Relationship between metabolic rate at 5 °C and body weight for six species of Perognathus.

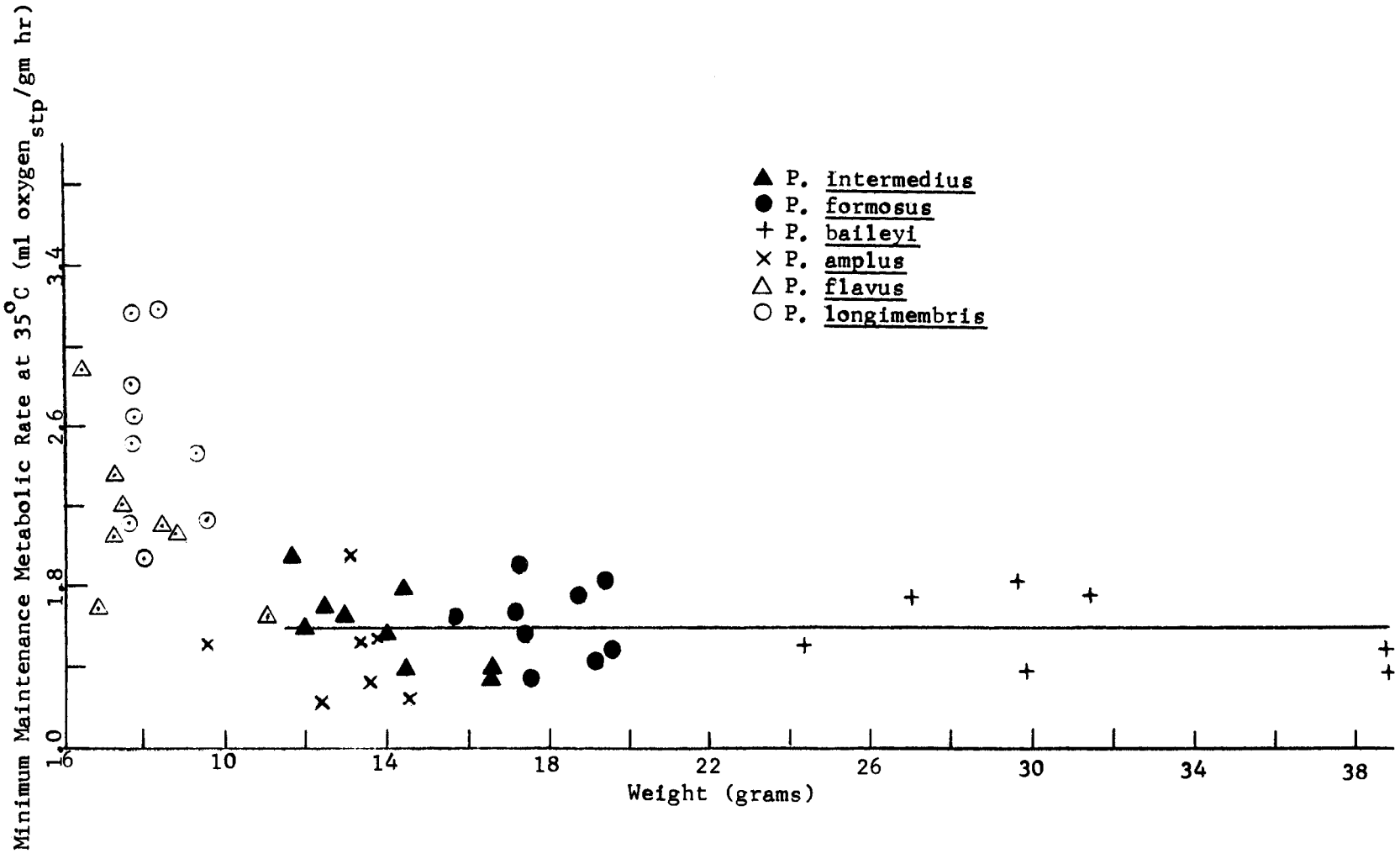

Figure 11. Relationship between minimum maintenance metabolic rates at 35 °C and body weight for six species of Perognathus.

Morrison et al. (15) for the Masked Shrew, Sorex cinereus (body wt. 2.9 to 4.0 g).

The lower critical temperature of a mammal is the lowest ambient temperature at which it can maintain normal body temperature by means of its minimum heat production and maximum insulation (i.e., no chemical heat regulation). The data of figure 9 indicate that the critical temperature is near 29.5°C for P. baileyi and P. formosus, 31.5°C to 32°C for P. intermedius, P. amplus and P. flavus, and 35°C for P. longimembris. Since the metabolic rates indicated by the horizontal lines in figure 9 are greater than minimum or basal rate, the true critical temperatures should be somewhat higher than these values.

The lower critical temperature is a function of maximum insulative value of the pelage and the level of the basal metabolic rate. In general, insulative value of mammalian pelage decreases with body size (22), and larger species thus tend to have lower critical temperatures. The present data, with the exception of those for longimembris are in agreement with this relationship.

The data for P. longimembris are an interesting divergence from the family of curves: the conductance of this species is less than expected (i.e., its insulation is greater); its minimum maintenance metabolic rate is the highest of the six species, and consequently its critical temperature is highest; and, a projection of the regression line intersects base line at 45.5°C, a point probably well above fatal body temperature for this species. The present regression curve for P. longimembris, based on nine individuals, is in close agreement with that previously calculated for a much larger group of measurements on this species (see Figure 7) so the observed difference is probably not a chance variation of measurements on this species.

A similar type of divergence has been noted for harvest mice (Reithrodontomys megalotis) by Pearson (18) and kangaroo mice (Microdipodops pallidus) by Bartholomew and MacMillen (2). These two small rodents showed conductances of 0.27 and 0.10 ml/g hr/°C respectively. The more effective insulation is an advantage at lower ambient temperatures, since it allows for less heat loss, but it is a disadvantage at temperatures near and above normal body temperature, since the individual then more easily becomes hyperthermic to the fatal point. Bartholomew and MacMillen (2) found kangaroo mice were killed by a few hours exposure to an ambient temperature of 39°C. In the present study, individual P. longimembris have died during short exposure to ambient temperatures of 37° to 39°C. Both P. longimembris and M. pallidus have very narrow zones of thermoneutrality, an obvious consequence of "exceptional" insulation.

That all of the very small mammals do not necessarily follow this trend is shown by the present data for P. flavus, and by the data of Morrison et al. (15) for the masked shrew (body wt. 2.9-4.0 g; minimum maintenance metobolic rate 9.0 ml/g hr; conductance 0.60 ml/g hr °C; critical temperature 22.5°C).

(2) Metabolic rate in relationship to body weight - The present study is of particular interest because it involves a group of closely related small mammals, two of which, P. flavus and P. longimembris are near the smallest size of mammal that exists. Only some insectivores are smaller, such as the masked shrew.

In the two curves of metabolic rate versus body size, figures 10 and 11, there is a sharp upward curve in metabolic rate, beginning at a body weight of about 10 grams. This break is more pronounced at 35°C than at 5°C. At 35°C mice ranging from 11.6 to 38.8 grams weight had the same average "minimum" maintenance metabolic rate. The individuals of the three lightest species, weighing less than 11 grams, had abruptly higher metabolic rates.

This situation has previously been noted by Pearson (17), who estimated that for small insectivores, the curve becomes asymptotic at about 2.5 gram body weight, which is then a theoretical lower limit to body size in mammals. The present curves for rodents appear to approximate this value. The size limit, at least in environments with low ambient temperatures, is determined by the maximum metabolic rate that can be maintained by the mammal, to balance an exaggerated rate of heat loss to the environment. Maximum rates of 29 ml/g hr were observed over periods of 1-2 hours for the masked shrew at 2°C (15). A rate of 10.8 ml/g hr has been observed for P. longimembris over a 24-hour period at 2°C (see Figure 7); a rate of 12.2 ml/g hr at 0°C is predicted from the regression curve (Figure 7) for this species.

(3) Comparison of physical conductances of different Perognathus - The data on metabolic rates of different species show that they have different total conductances ranging from 0.40 ml O_2/g hr/°C for 7.5 gm P. flavus to 0.18 ml O_2/g hr/°C for 30.6 g P. baileyi.

In order to study the physical conductances of several species, as separate from physiological factors influencing heat loss, the cooling rates of freshly killed individuals were measured. For these measurements, rectal and subcutaneous thermistors were implanted and the live animal was kept at the desired ambient temperature for several hours until body temperatures stabilized. Chloroform was then introduced into the chamber. The animal died quickly, usually without a change of posture. Core and subcutaneous temperatures were then recorded as the dead body, resting in a natural position, cooled to ambient. The log of the gradient, $T_c - T_s$, was plotted against time; the slopes of these lines give cooling constants in terms of degrees change in T_c per minute per degree of gradient.

Dead P. longimembris cool more rapidly than the two larger species, as is expected from geometrical relationships; P. longimembris also has the highest conductance. This could be due to pelt differences, since pelt thickness decreases with animal size (13).

Table 4. Physical Cooling Characteristics of Three Species of Perognathus

Species	Body wt.	Surface area (1)	Cooling Constant $\frac{\Delta T_c}{\text{min.}^{\circ}C}$	$\frac{\text{cal}}{\text{gm min.}}$	Conductanct, $\frac{\text{cal}}{\text{cm}^2 \text{ min}^{\circ}C}$	
P. longimembris	8.18g	17.82cm^2	0.0362	0.030	0.0138	0.0136
	8.14	17.76	0.0350	0.029	0.0133	
P. formosus	21.70	34.26	0.0210	0.017	0.0110	0.0116
	19.68	32.09	0.0239	0.020	0.0134	
	19.51	31.90	0.0204	0.017	0.0104	
P. californicus	31.68	44.14	0.0189	0.016	0.0113	0.0118
	28.18	40.81	0.0210	0.017	0.0120	
	25.68	38.35	0.0219	0.018	0.0122	

(1) Calculated as: $\text{cm}^2 = 9 \text{ wt (gm)}^{0.67}$

The data are not extensive enough to judge whether the conductance of P. longimembris is as high as to be expected in relation to its smaller size.

Heat loss rates of these species of Perognathus in terms of cal/gm hr $^{\circ}C$, are similar to those reported by Morrison and Tietz (14) for two species of Alaskan rodents in the same body weight range. This suggests that Perognathus may have a more insulative pelage than expected for temperate zone rodents.

Comparison of Metabolic Rate in Air and 80 - 90% Oxygen:

Procedure - The possibility that the high oxygen atmosphere used in the Metabolor influenced metabolic rate was assessed in several ways:

(2) Metabolic measurements were made on two groups of four mice each, subjected simultaneously to 90% oxygen and air; at intervals of 2-4 hours the group treatments were reversed. The differences between rates for the same animal in oxygen and air were tested statistically. Test were run at 5$^{\circ}C$ on P. longimembris, P. fallax, P. formosus, P. inornatus, and at 15° and 25° on P. longimembris.

(2) Two groups of four P. longimembris were kept at 25$^{\circ}C$ for 4 days in air or oxygen (no reversal of groups). The differences between groups was tested for significance, as well as differences with time within groups.

(3) Measurements were made on P. longimembris kept in an atmosphere of air over a range of ambient temperatures. These data were compared with previous data of measurements made in 80 - 90% oxygen.

Results and discussion - None of the seven comparisons, in which the same animals were alternated between air and oxygen, showed significant differences. Since

the effects of oxygen poisoning on laboratory mammals are accelerated by speeding up the metabolic rate, the lack of differences in the tests at a T_a of 5°C are the best evidence for no effect of high oxygen atmosphere, at least during the duration of the exposure.

The four-day test was made at 25°C, so that there would not be a problem of the mice becoming torpid and avoiding the situation. The air and oxygen groups were not significantly different at any time, nor was there any significant change in metabolic rate with time.

Figure 12 shows the data for P. longimembris in air versus 80 - 90% oxygen, over a range of ambient temperatures. Metabolic rates in the two atmopheres are not significantly different. Similar results were obtained in air-oxygen comparisons for P. formosus and P. inornatus.

On the basis of the tests that have been made thus far, it is reasonable to conclude that the procedure of measuring metabolic rates with the Metabolor is probably satisfactory and that the measurements are equally valid for animals in an atmosphere of air.

Oxygen Poisoning in P. longimembris:

Since the atmosphere in the Metabolor is not "pure" oxygen, several brief tests were run with animals in an atmosphere of 99.5 - 100% oxygen, to see if this species is exceptionally tolerant to oxygen poisoning.

Fourteen P. longimembris were kept continuously in dry, pure oxygen at atmospheric pressure and T_a = 22°C; 10 animals were similarly kept at 10°C. In both groups the first deaths were observed at 74 hours (3 days); 50% mortality occurred by 95 hours (4 days) in the 10° group and 98 hours in the 22° group. In almost all animals that were autopsied, the lungs were filled with bloody fluid; the other internal organs were grossly normal. Some mice also had bloody fluid within the auditory bullae.

Therefore, P. longimembris shows the same symptoms of pulmonary edema as small laboratory mammals exposed to pure oxygen at one atmosphere. Their survival time is probably not any longer than laboratory species. In 95 - 99% oxygen guinea pigs die in 3 - 6 days (9); some white rats die within 45 hours in 90% oxygen (3).

Pulmonary edema was noted in some P. longimembris that were kept more than a week continuously in 80 - 90% oxygen in the Metabolor. Therefore, oxygen poisoning is a possible complication in all such long term runs that have been made. It is necessary to measure survival times in atmospheres of 70, 80 and 90% oxygen, to asses the extent of this complication.

Hypometabolic states in P. longimembris:

General remarks - As previously shown by Bartholomew and Cade (1), P.

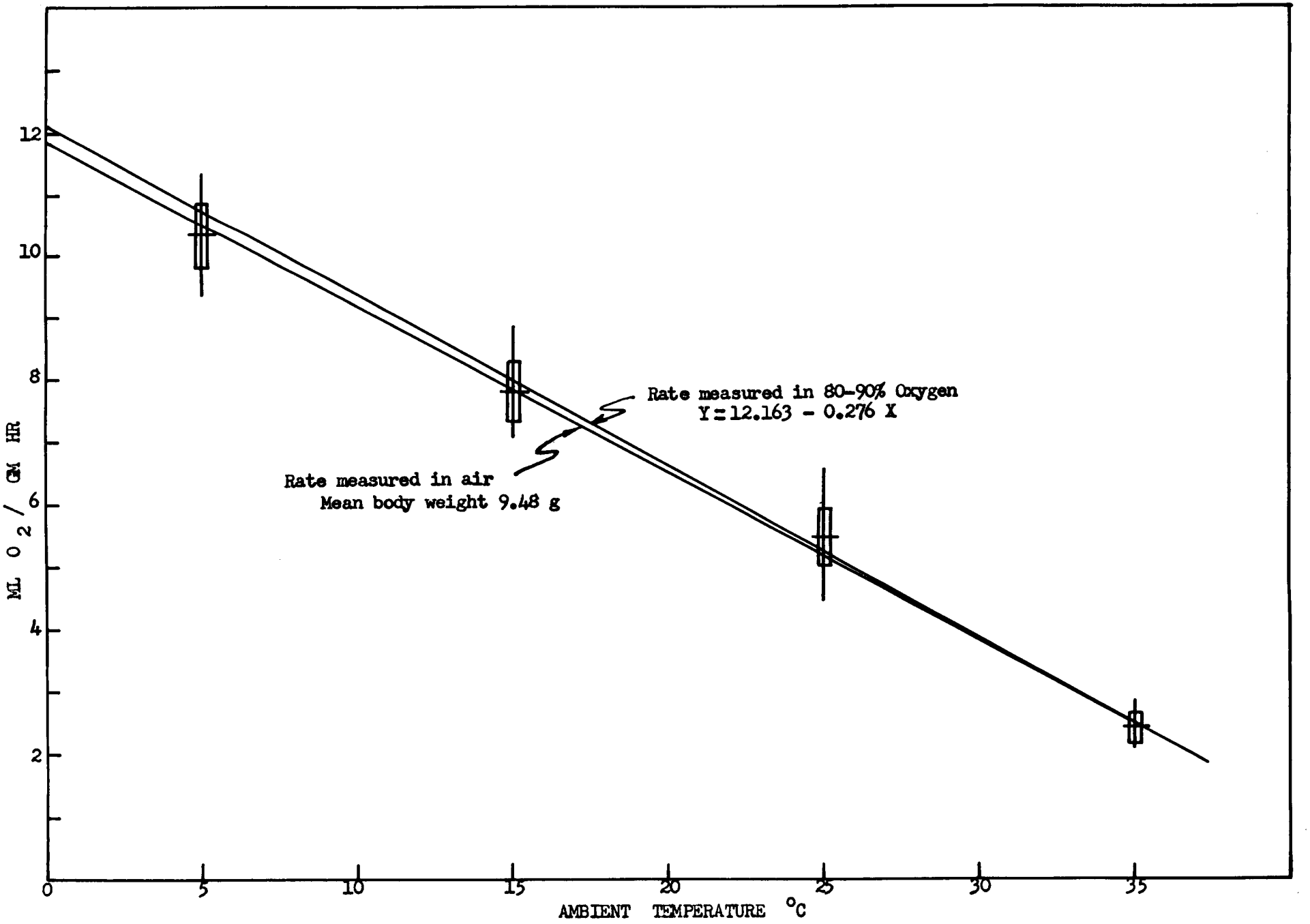

Figure 12. Maintenance metabolic rates of P. longimembris as measured in air and 80 - 90% oxygen atmosphere.

longimembris can be induced easily to become torpid by depriving them of food and/or reducing the ambient temperature. Sometimes animals become only semitorpid, and while their body temperature decreases considerably, it is still maintained above ambient; the present authors prefer the term hypometabolic state for these situations. Often, however, deep body temperature drops to very near ambient temperature, incident to a deep hypometabolism with metabolic rate less than 0.10 ml O_2/gm hr; since these animals can spontaneously rewarm, they can be said to be in a state of hibernation.

The very reduced metabolic requirements of hibernating mammals are attractive from the point of view of planning experiments in space with live animals. Hence, special attention was given to the study of the physiology of P. longimembris during induced periods of hypometabolism, and to the incidence and duration of torpor.

Minimum rates of metabolism in deep hypometabolic states - Table 5 summarizes the results on minimum metabolic rates for P. longimembris that were deeply hypometabolic one or more times during metabolic measurements that extended over a period of several days. From the several temperature records that were made simultaneous with metabolic measurements, it seems quite certain that the body temperatures of all these animals were very near ambient temperature at the time of the minimum metabolic rates. (Figure 13)

Kayser (12); (see pp. 32-103) summarizes data on metabolism of various mammals, mostly rodents, in states of "deep hibernation". A variety of these are reported to consume 0.02 to 0.03 ml O_2/gm hr in "deep hibernation". In deep hibernation, metabolism is nearly proportional to body weight, according to the relationship: Kcal/24-hrs = 1.97 $kg^{0.94}$. According to this formula, a 9-gram mouse should use 0.025 ml/gm hr. As shown in Table 5, P. longimembris measured in an air atmosphere did reach minima of 0.017 ml/g hr (this for one animal, item 1, Table 5) and 0.0268 ml/gm hr, as the average for a group of 6 mice measured collectively (item 2).

For the pocket mice that were measured in the Metabolor, the lowest individual rate was 0.0404 ml/g hr for one animal out of eight measured in air and in the dark (item 3); all other measurements are for animals under normal photoperiod. Very close to this is a minimum of 0.042 ml/g hr for one animal out of eight measured in an atmosphere 85 - 90% oxygen (item 5b).

Since the three lowest values are all for mice in air, it is quite possible that a high oxygen atmosphere may stimulate metabolism in these deeply hypometabolic states (as it is reported to stimulate metabolic rate of normothermic white rats).

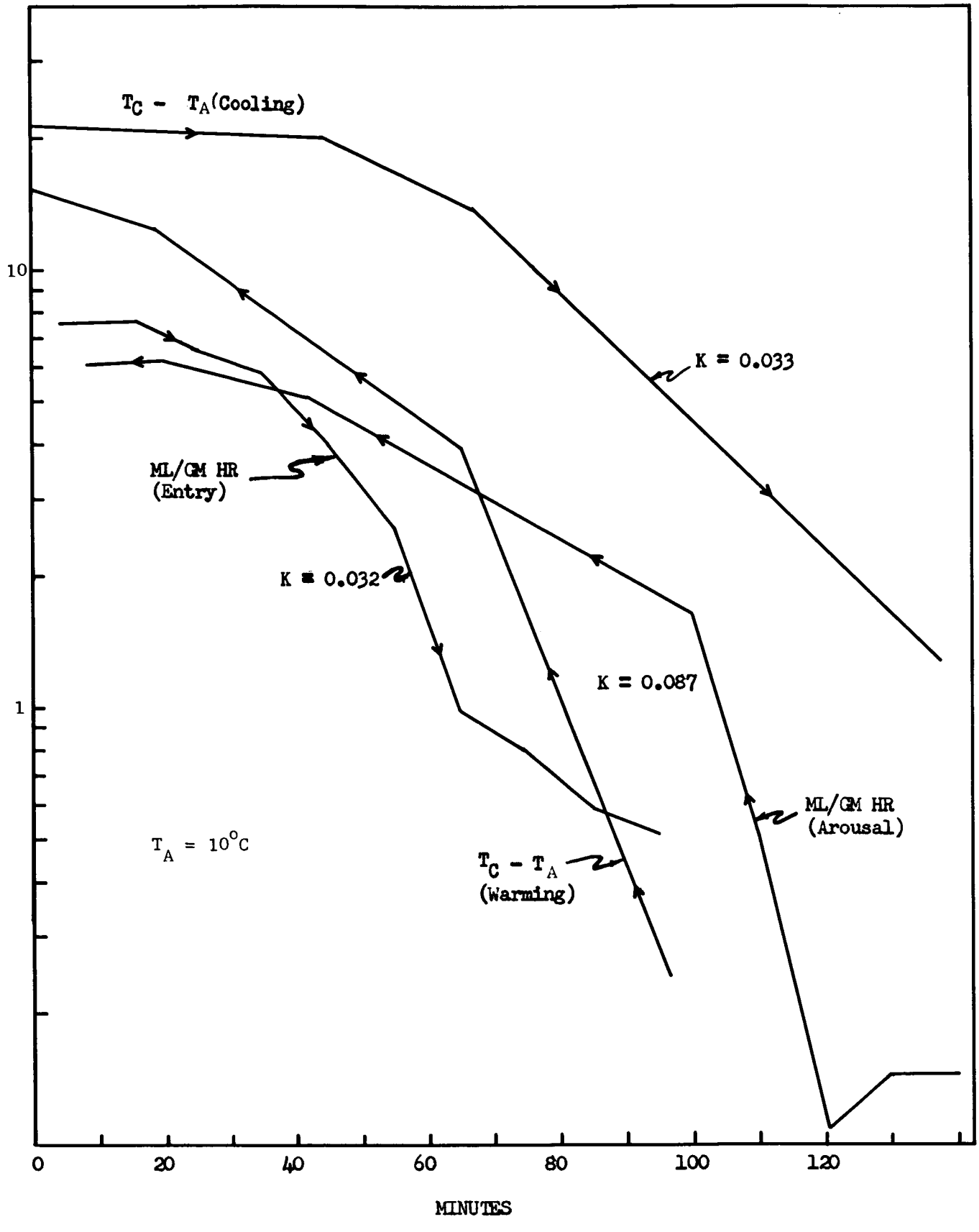

Figure 13. Metabolic rates and body temperatures during entry into and arousal from periods of deep hypometabolism.

Table 5. Minimum Rates of Metabolism of P. longimembris in deep hypometabolic states

	Conditions	Average minimum	Range of individual minima
1.	10°C, group of 6 mice measured collectively in air (Beckman P.O.A.)	0.0268	----------
2.	10°C, one mouse with implanted thermistors, in air (Beckman P.O.A.)	0.017	----------
3.	10°C, 8 mice, in air, fasting, in dark, (Metabolor) Aug. 31, 1963	0.0895	0.0404-0.121
4.	10°C, 9 mice, in 80-90% oxygen, fasting, (Metabolor) Sept. 27 - Oct. 2, 1962	0.122	0.090-0.148
5a	5°C, 7 animals, 85-90% oxygen, fasting, (Metabolor) Aug. 21-24, 1962	0.192	<0.148-0.269
5b	10°C, 6 animals of same group, Aug. 22-24, (Metabolor) fasting	0.224	0.159-0.395
6	15°c, 6 animals, 85-90% oxygen, fasting Nov. 6-9, 1962	0.0986	0.042-0.168
7a	15°C, 8 animals, 85-90% oxygen, fasting during and 3 days before run. (Metabolor) Aug. 27-29, 1962	0.184	0.153-0.216
7b	22°C, same group, Aug. 29-31	0.371	0.320-0.482

The other minima are seven to twenty times greater than the deep-hibernation values of Kayser. This is probably due to environmental conditions which disturbed the animal. Kayser (12); (see p. 97) mentions the difficulty of obtaining values in the range of 0.02-0.03 ml/g hr for hamsters, until special precautions were taken to avoid disturbing the animals. Otherwise the minima he obtained were 10 times greater than 0.02-0.03 ml/g hr. Methods are also a factor in measurement of minima; the two lowest values obtained for P. longimembris were from measurements with the Beckman Paramagnetic Oxygen Analyzer (items 1,2 Table 5), which is more adaptable to detection of minimal rates than the Metabolor.

Q_{10} for metabolic rate of P. longimembris - Values for Q_{10} can be calculated in two ways from the data of table 5, and figure 8: (1) from measurements on animals that have been deeply hypometabolic at two temperatures successively, such as items 5a and 5b for animals at 5° and 10°C successively, and 7a, 7b at 15° and 22°C successively; (2) from the minimum metabolic rates of deeply hypothermic mice and the basal metabolic rates of normothermic animals. In both cases, Q_{10} is calculated

as: $Q_{10} = \left(\frac{k_1}{k_2}\right)^{\frac{10}{t_1 - t_2}}$

where k_1 and k_2 are the metabolic rates at temperatures t_1 and t_2.

The average minimum rates for data of items 5a and 5b give a Q_{10} value of 1.40; the average minimum rates for data of items 7a and 7b give Q_{10} = 2.73, while the absolute minima give Q_{10} = 2.89. These value are close to that of 2.2 for isolated tissues of hibernating ground squirrels (13).

The Q_{10} figured from a minimum hypometabolism of 0.0268 ml/g hr at 10°C (item 1, table 5) and a basal metabolic rate of 1.130 at 35°C (Figure 8), gives a value of 4.5. Although such calculations are reported in the literature, they are illogical in that they utilize one value for "cold-blooded" nonregulating mice and another for basal, but still "warm-blooded" and regulating animals. The fact that the value of 4.5 is twice as high as the Q_{10} values based on only "nonregulating mice" indicates that the former involves regulatory components. The Q_{10} of 4.5 does exceed the theoretical value of 4.0 which was calculated by Morrison (13) to be necessary for the survival of a small hibernating mammal.

Table 6. Metabolic "Costs" and "Savings" of P. longimembris During Periods of Hypometabolism

Conditions of Experiment	Percentage of time		Percentage of total metabolism during	
	Normo-metabolic	Hypo-metabolic	Normal periods	Hypothermic periods
1. Pearblossom group, 9 mice, 8.21 g. ave. wt. 10°C, 1 gm seed at start, 80-90% oxygen in Metabolor, normal photoperiod. Sept. 27-Oct. 2, 1962	37.5	62.5	94.2	5.8
Actual metabolism averaged 33.19% of predicted (28.5-43.7%)				
Hence, metabolic saving 66.81% of expected (56.3-71.5%)				
2. Whitewater group, 8 mice, 9.82 g. 10°C, fasting, in air in Metabolor, continuous darkness July 31-Aug. 7, 1963	19.1	80.9	89.9	10.1
Actual metabolism averaged 19.64% of predicted (5.0-37.4%)				
Hence, metabolic saving 80.36% of expected (62.7-95.0%)				

Metabolic requirements during periods of hypometabolism - As shown in figures 15 - 21, P. longimembris does not undergo prolonged or sustained hibernation under the experimental conditions tested thus far. Individuals arouse usually every day when kept in a high-oxygen atmosphere and less frequently in an air atmosphere. Even with arousal every one or two days, there is still a considerable metabolic saving of about 65 to 80% of the cost of sustained normometabolism. Data bearing on this are given in Table 6 .

The second group of animals in Table 6 was chosen for heavy-weight individuals, and was kept in continuous darkness; both of these conditions were expected to enhance the occurrence of prolonged torpor. In addition, the second group was kept in air, rather than 80 - 90% oxygen.

The individual mice in the group in air did have fewer arousals; they were hypometabolic for a greater part of the time, and consequently had a greater metabolic savings.

This comparison, and other data, from runs with the Beckman P.O.A. strongly suggest the possibility that a high-oxygen atmosphere is somewhat stimulatory and prohibits the pocket mice from going into prolonged or sustained deep hypometabolism. Further tests are needed to assess this.

Spontaneous hibernation of P. longimembris kept at 10°C but not deprived of food - In nature, P. longimembris "disappears" during the late fall and winter months, and cannot be live-trapped. However, in the spring, marked animals are recaptured at or near the trapping station where they were last captured 5 to 7 months earlier (5). The obvious conclusion is that the mice are staying below ground during this time. Whether they are undergoing prolonged hibernation, or merely avoiding exposure outside their burrows is not known. Some data of the present study bear on this point.

(1) Behavior of mice in artificial burrow systems.

Procedure. Artificial burrow systems were constructed for monitoring the metabolic state of pocket mice while they were "underground". Each burrow consisted of a wide-mouth vacuum bottle (nest chamber), containing a thermistor and connected to a plastic pipe (runway) extending to the surface. The nest and runway were buried in sand in an aquarium kept in a coldroom at 10 $\pm$ 0.5°C. Cotton nesting material and mixed seeds were supplied. All animals tested had previously been kept at 10°C for several months in gallon jars. When the mice were active, the temperature in the vacuum bottle was warmed well above ambient; when the animal was torpid, or outside the burrow, the nest chamber temperature fell to ambient. Each morning the sand surface in the aquarium and the food supply were

inspected for evidence that the animal had been active outside its burrow; after inspection the sand was smoothed and food was added, if necessary.

Results - Of the four animals tested simultaneously in the aritifical burrow systems, one was regularly active "above-ground". During a 33-day period this pocket mouse was active every night for about four hours. For 16 days the animal became torpid during the day in its burrow.

The other three mice characteristically remained normometabolic but stayed within their nest chamber. One animal remained "underground" for three weeks continuously.

(2) Incidence of spontaneous torpor at 10^{o}.

Procedure. A group of 64 P. longimembris was kept in a cold-room at 10^{o}C over a period of 7 months (Dec. - July 1963). Animals were kept in gallon jars with sand and nesting material. Mixed seed was provided in surplus. The photoperiod was kept constant at 0800 to 1700; illumination was by only a single flourescent tube. Each morning at 0800-0900 a record was made of all torpid animals. All animals showing arhythmic breathing, periodic apnea and sluggish response to stimuli, were classified as torpid. Numerous measurements of deep body temperatures (T_c) were made on torpid animals, and in all cases T_c was less than 1.5^{o}C above ambient.

Results - Temperature in the cold room was initially set at 15^{o}C, and for two weeks no torpid animals were observed. The temperature was then reduced to 10^{o}C, on Dec. 10, 1962; no torpid animals were present until Jan. 2, 1963. In figure 14 are plotted the percentages of animals torpid, in the morning, from January through July 1963. There is a general upward trend of mean monthly values from about 11% in January to 21% in July. Because of the extreme range of values (from 0% on one day in March to 39% in June) these monthly means are probably not significantly different.

Table 7 summarizes the frequency of torpidity in the experimental group for observations made on a total of 140 days. The median is an incidence of torpidity of 11 to 15 times during 140 days; 17% of the animals were never torpid when observed in the morning while only 1% were torpid as many as one third of the days.

Table 7. Frequency of Torpidity in Experimental Group Over 140 Days of Observation

Number of times observed torpor	No Torpor	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50
% of total group	17.1	26.9	19.0	11.4	3.7	6.7	4.5	3.4	0.8	4.9	0.8

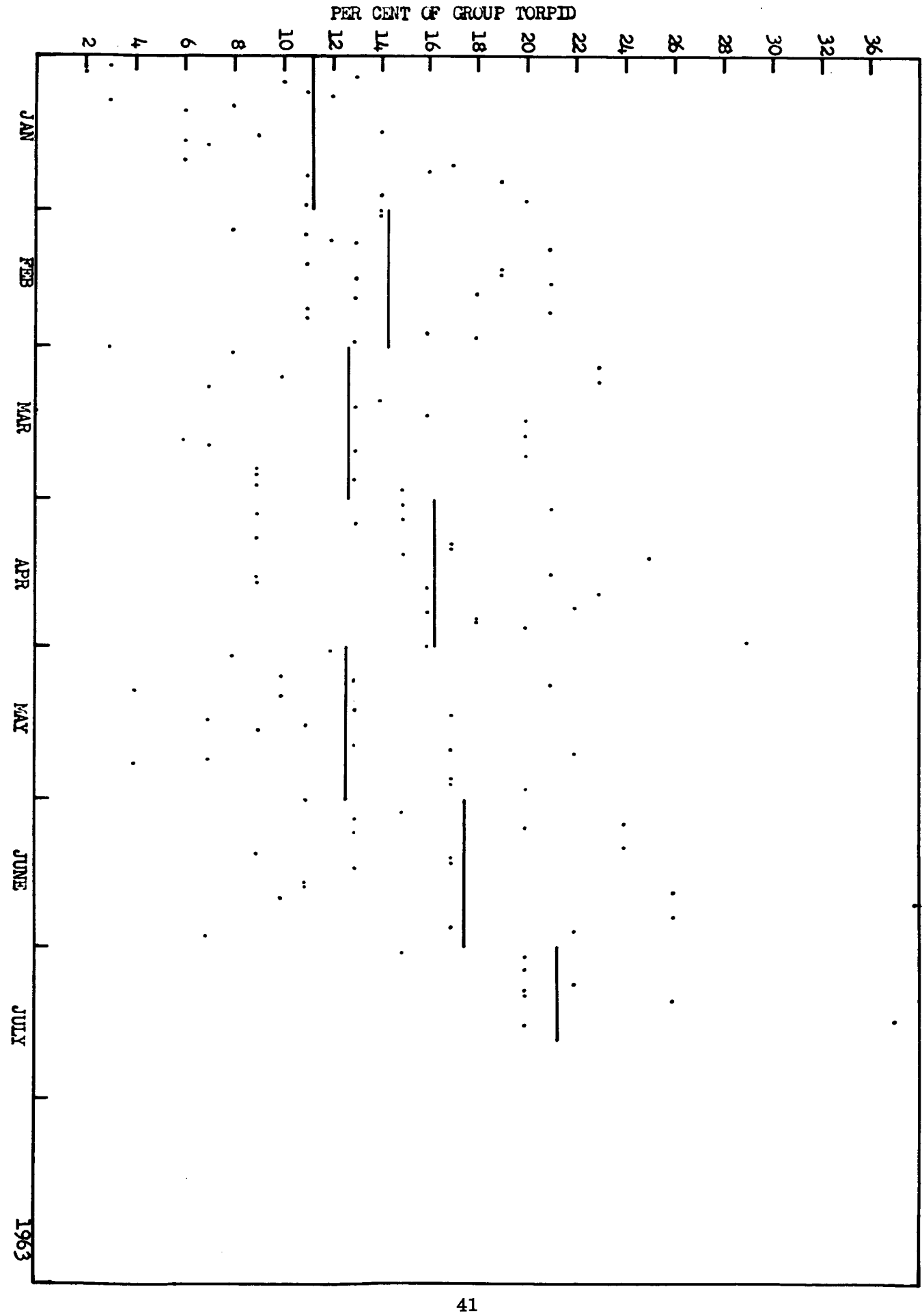

Figure 14. Incidence of torpidity of P. longimembris kept at T_A = 10 °C but allowed food.

On several occasions animals that were observed to be torpid in the morning were then observed at hourly intervals throughout the day. The general pattern was that these animals remained torpid until late afternoon, when they began to arouse. Only rarely did an animal that was not torpid at 0800-0900 become torpid later in the day.

The records of animals that showed the higher frequencies of torpidity, 31 to 50 times, were examined as to the sequence of torpor periods. These data indicate that there are periods of greater incidence of torpor; each of the six animals examined in this regard showed a high incidence only during a 3.5 month period. However, the onset of this period varied from mid-January to the beginning of April. The periods of all animals overlapped in April.

Discussion. When food is available, P. longimembris shows only a low incidence of torpidity at a T_a of 10°C under the present experimental conditions. The majority of animals apparently avoided the hypometabolic state while only a few became torpid with any regularity. The duration of the period of highest incidence of torpor (extending about 3.5 months) falls within the period when this species has been observed to disappear in nature (5). The trapping records of the Radioecology Section of Nuclear Medicine and Radiation Biology, UCLA, also show no P. longimembris trapped from late November until March.

These present data, and those on animals with artificial burrow systems suggest that P. longimembris is not a long-term hibernator, but spends a major portion of their "winter disappearance" underground, normothermic or alternately normothermic and hypothermic. Assuming that the figures of Table 8, group 2, apply in nature, a two-month supply of stored seeds would allow a pocket mouse to stay underground continuously for at least 6 months, if it aroused only every other day or so to feed.

As shown by other experiments, pocket mice deprived of food show a higher incidence of torpidity, and may remain torpid 4-5 days or longer. However, the absence of food is not to be expected in nature in a species with a foraging and food-storing behavior.

Conditioning effect of prolonged exposure to cold - Animals that showed a high incidence of torpor during their extended stay in the cold-room at 10°C were thought to be conditioned perhaps to the process of entry and arousal from hibernation, and hence able to better survive subjection to 10°C without food. Three animals that showed high incidence of torpor were compared with three others that had also been in the cold room, but had never been observed torpid. The animals were placed in vacuum bottles with sand, but without food. The temperatures within the bottles were recorded

Table 8. Torpidity and Survival of Two Groups of P. longimembris at 10°C; Those Never Demonstrating Torpidity Previously Versus Those That Had Frequently Been Torpid.

Group	Surv. after 7 days	Surv. after 11 days	Surv. after 14 days	% Original Wt. after 7 days	% Original Wt. at end exp.	Longest torpid period	Time before 1st torpid period	# Days in exp. chamber	Total No. Arousals
Never torpid previously	3	2	0	81.2	Total - 79.9* surv. - 79.2	2.5 days	2.5 days	11	11
Frequently torpid	3	2	1	83.4	Total - 73.5** surv. - 62.2	2.5 days	1.0 days	14	13

* 1 Dead

** 2 Dead

continuously for 14 days. Animals were weighed on the 7th day and returned to the bottles.

The results are summarized in Table 8. The "frequently torpid" mice did become torpid earlier, and showed a higher survival and a greater tolerance of weight loss. The data suggest a conditioning effect, but are not numerous enough to establish this point.

Metabolic rhythms in Perognathus:

General remarks - In the course of establishing metabolic base lines for pocket mice, as previously reported here, numerous data have been obtained which strongly suggest the presence of a biological rhythm in P. longimembris which is about 24-hours long (i.e. a circadian rhythm). The research performed to date was not designed to study or test for the presence of circadian rhythms, and as a result obvious gaps in the experimental data exist. It is significant, however, that despite variations in food supply, ambient temperature, atmospheric composition, photoperiod, and gamma-irradiation stress, what seems to be a biological rhythm has persisted. As will be developed later, if rhythms are established as existing in this species it is a very promising situation for experiments involving the placing of organims in earth-

orbits and testing the question of the influence of exogenous cues on biological rhythms.

Circadian rhythms have been shown for numerous rodents and other mammals under normal environmental conditions as measured with activity wheels (7,19), body temperature (8), and rate of metabolism (18).

Perognathus is unusual in the depth to which its metabolism can drop during its resting periods. Even when food is available and environmental temperatures are moderate, pocket mice frequently allow their rate of metabolism to drop below the minimum needed to maintain a normal mammalian body temperature as in figure 1. (This is referred to as hypometabolism in the present report.) When pocket mice are starved and temperatures are low, they allow their metabolic rate to drop every day to near a level equal to that found in hibernating mammals. (This is referred to as deep hypometabolism in the present report.) At such times, a mouse's internal temperature approximates that of his environment.

It has been known for a long time that similar daily metabolic fluctuations occur in bats (10), and more recently there have been reports for the Birch Mouse (11).

The factors responsible for circadian rhythms in mammals are only partly understood, especially the endogenous and subtle exogenous factor. This topic is considered in Cold Spring Harbor Symposium (25(1959)) on "Biological Clocks" which summarizes much of the literature. The following examples indicate the existence of a circadian rhythm in pocket mice as evidenced by measurement of oxygen utilization.

Response to moderate normal environmental conditions - Most P. longimembris, when they are kept individually in 1-liter beakers at a moderate temperature (22-24°C), show obvious alternating periods of high and low oxygen consumption (see individual A in figure 15). Even when food is provided, some individuals become hypometabolic (see individual B in figure 15). At moderate temperatures and with food present, deep hypometabolism occurs with a low indicence (see Groups 1 and 2 in Table 9).

Effect of factors experimentally varied from normal -

(1) Withdrawal of food at 22°C ambient (see Group 3 in Table 9). Starvation accentuates the amplitude of the metabolic rhythm, with animals becoming deeply hypometabolic every day (see Figure 16). There is not an exact coincidence of low points of individual animals at moderate temperatures, but there is general agreement as to the lengths of the cycles of individual mice as shown in the integrating bar graph, Figure 17.

The incidence of torpidity in the Little Pocket Mouse when starved at moderate temperatures may be 100% (Group 3, Table 9).

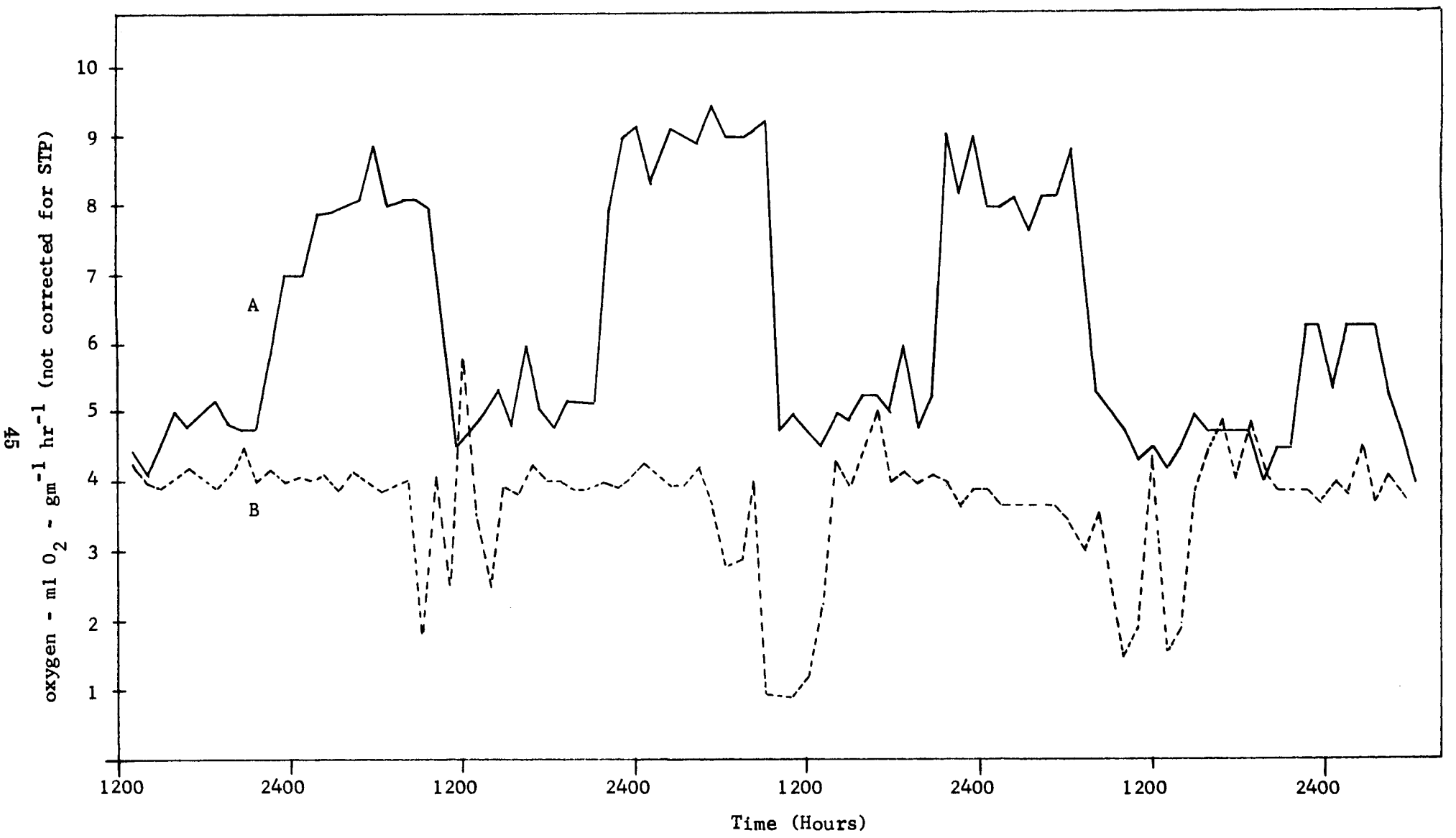

Figure 15. Metabolic activity of 2 representative P. longimembris maintained at 24°C with food.

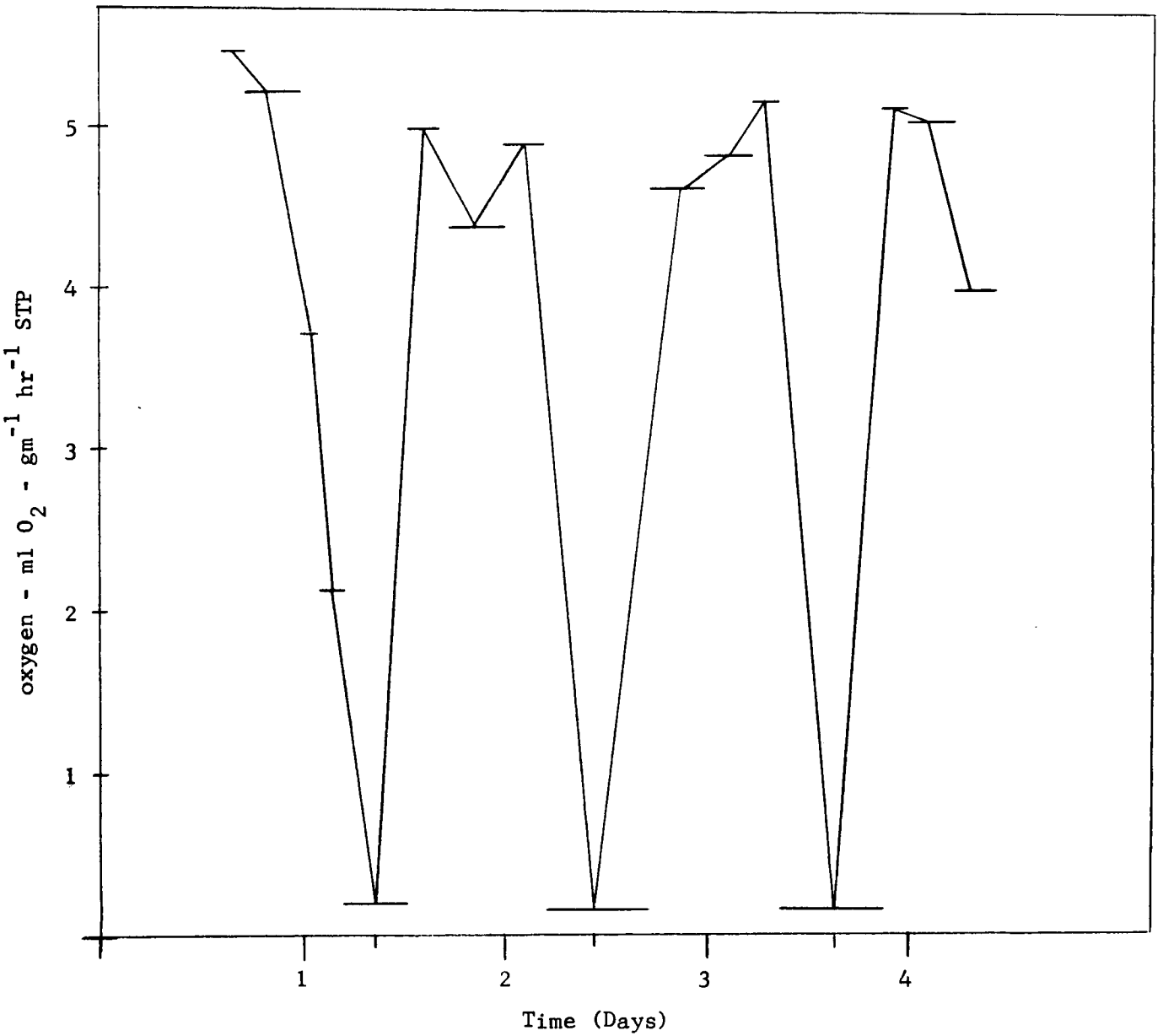

Figure 16. Metabolic activity of a single P. longimembris maintained at 22°C with no food.

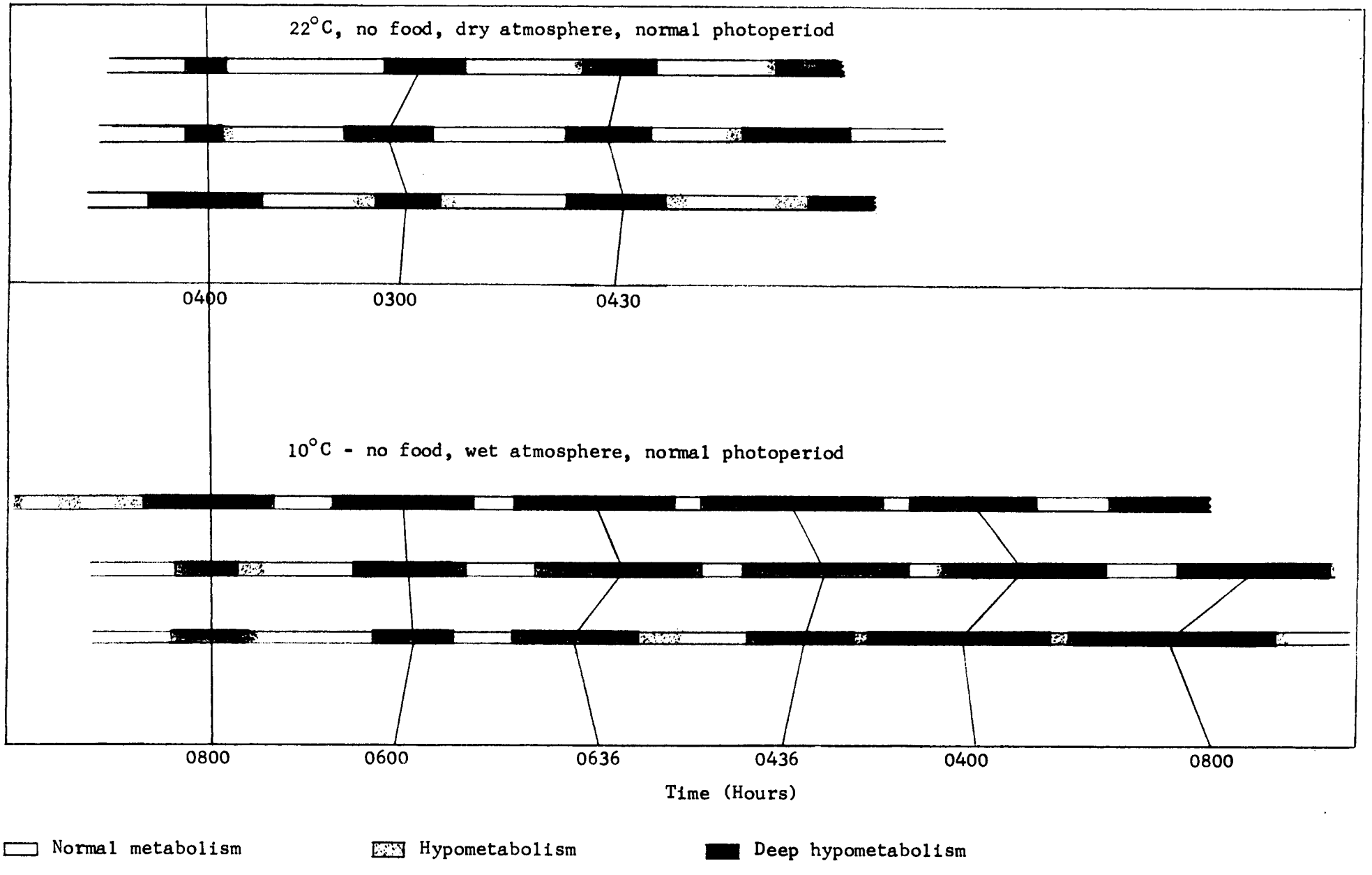

Figure 17. Comparison of rhythm of hypothermic periods in two groups of three P. longimembris studied under different environmental conditions.

(2) Withdrawal of food at 10°C ambient (Group 4, Table 9). Individually housed mice starved at 10°C showed an accentuated rhythm, as at 22°C. there was somewhat greater coincidence of times of midpoints of deep hypometabolism (Fig. 17), compared to the 22°C group, and the periods of deep hypometabolism are much more prolonged. (Compare dark bars in Fig. 17). Several mice remained deeply hypometabolic for two or three days in succession , but this is unusual in mice kept isolated from other individuals.

Grouped individuals under the same conditions demonstrate a group metabolic rhythm, as in figure 18. Here the majority of animals show a 24-hour rhythm beginning with the metabolic low on the second day. A secondary 24-hour rhythm, which begins late in the third day, may represent one or more animals that are "out of step."

(3) Animals in darkness. (Groups 6 and 7 in Table 9) The metabolic rhythm of groups of P. longimembris (Group 6, Table 9) and also P. inornatus (Group 7, Table 9; Figure 19) persists in continuous darkness, at least for 7 days.

Periodic arousals are not eliminated by subjecting the animals to an atmosphere which is 4.5% carbon dioxide (Group 6, Table 9).

Two mice were kept individually in a dark, sound proof box for 11 days. One animal showed an unusually precise 24-hour rhythm, the other showed an unusual, but precise, 48-hour rhythm (Fig. 10).

(4) Effect of acute radiation (Group 8, Table I). P. longimembris is able to survive radiation dosages that are fatal to other mammals (see Part II, Radiobiology). Nine mice, which had been subjected to 1400-roentgen dosage, were shortly thereafter placed within a normal enivronment for metabolic measurements (Fig. 21). Metabolic rhythms were demonstrated in all individuals. The rhythm differed from controls only in a greater incidence of periods of deep hypometabolism (compare Groups 2 and 8 in Table 9).

Some of the irradiated animals had a hypometabolic sequence that is very similar to the food deprived groups (compare Fig. 21 and Fig. 16); however, most of this group, after an initial loss of 1-2 g during the first week, gained weight during the second week of the study.

The irradiated animals were disturbed on the seventh day for weighing and examination and the atmosphere in the Metabolor was then re-established at 90% oxygen. After this disturbance there was an average shift of 4.3 hours (6.0 - 0.3) of the midpoint of deep hypometabolism towards later in the day. No such shift was observed in non-irradiated animals disturbed in the same way (Table 9, Group 2). Six animals were restudied 79-83 days after irradiation. The rhythms

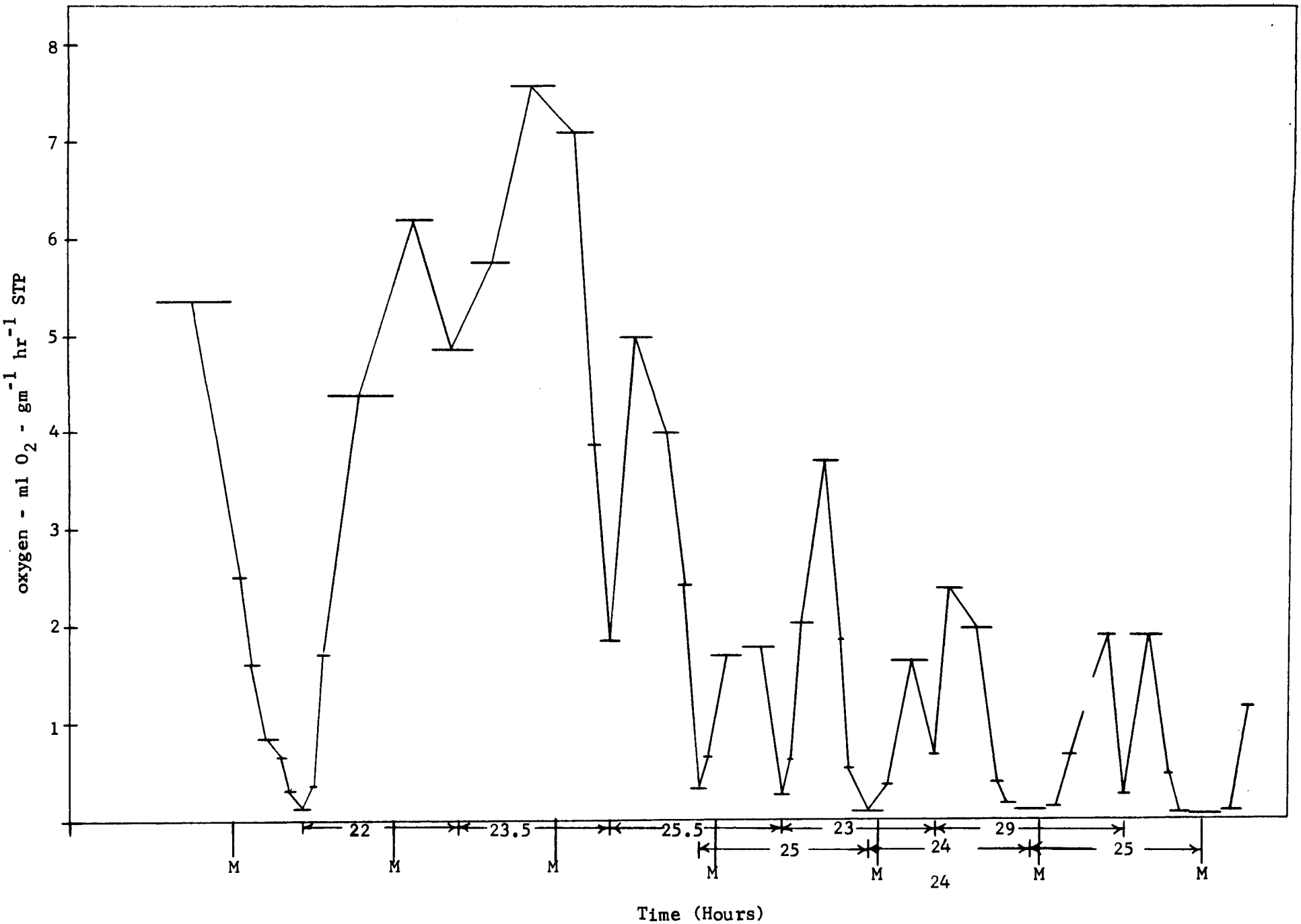

Figure 18. Metabolic activity of 6 P. longimembris monitored together, normal photoperiod at 10°C.

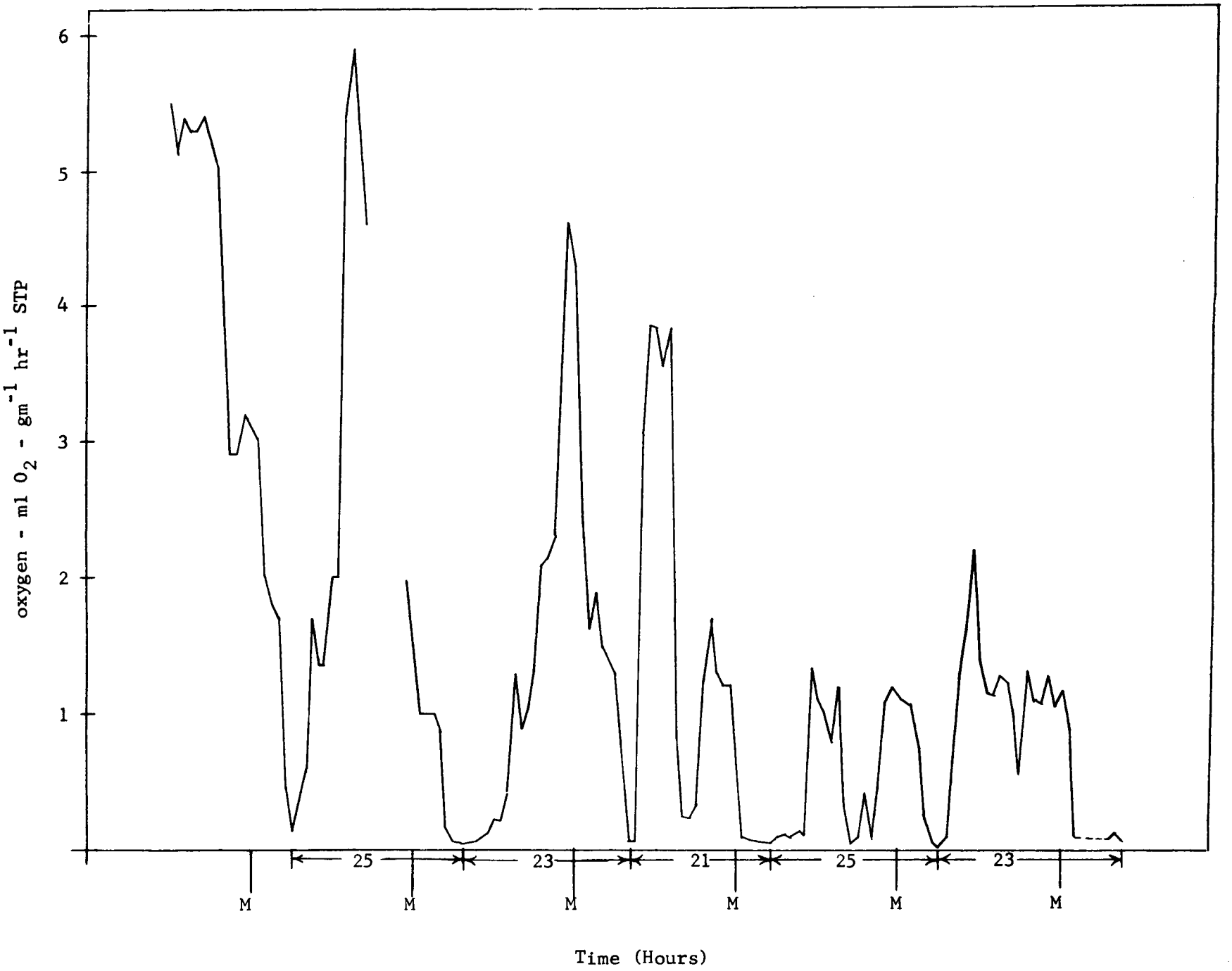

Figure 19. Metabolic activity of 6 P. inornatus monitored together in dark chamber at 10°C.

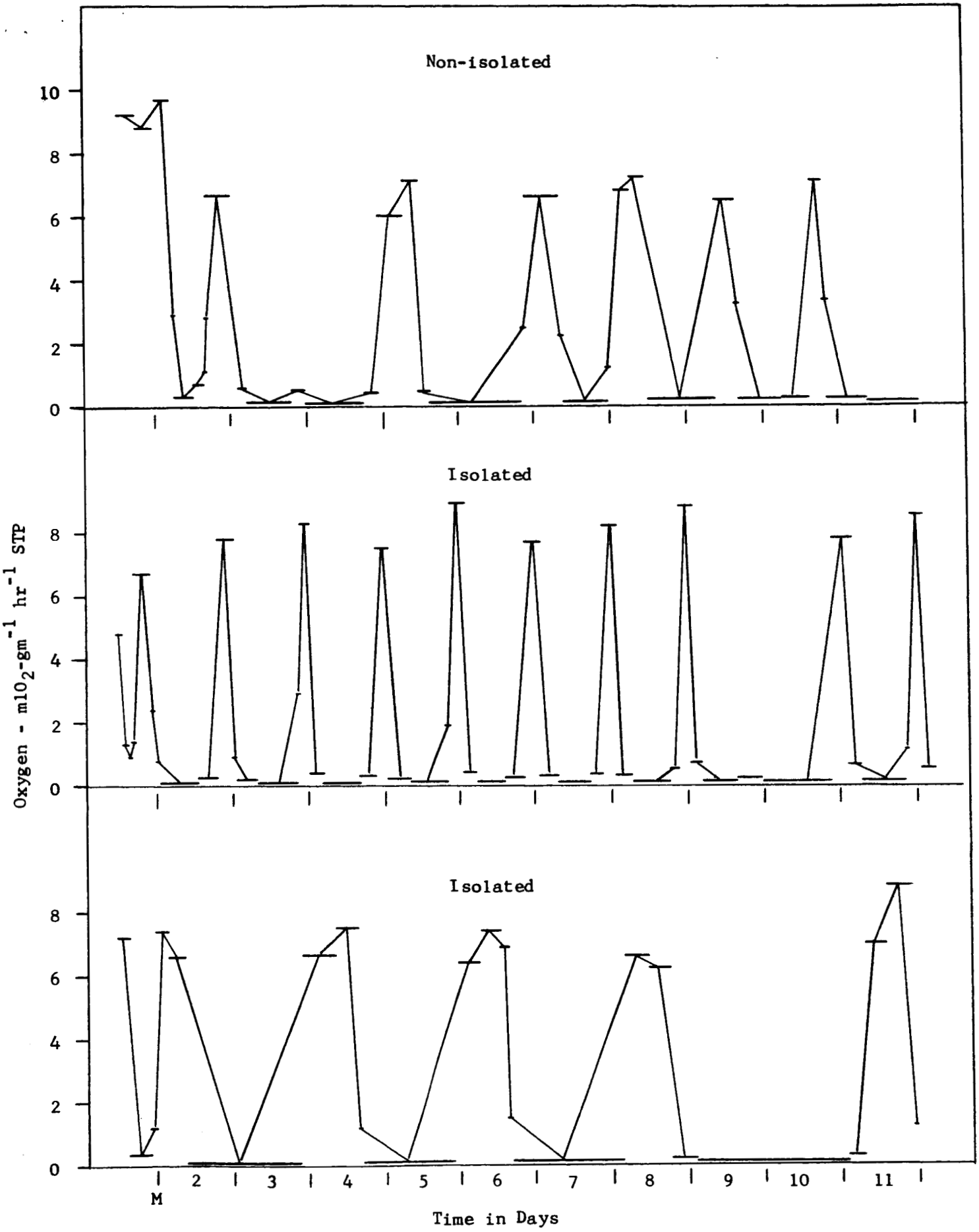

Figure 20. Relative metabolic activity of 3 P. longimembris in "isolated" and "non-isolated" chambers.

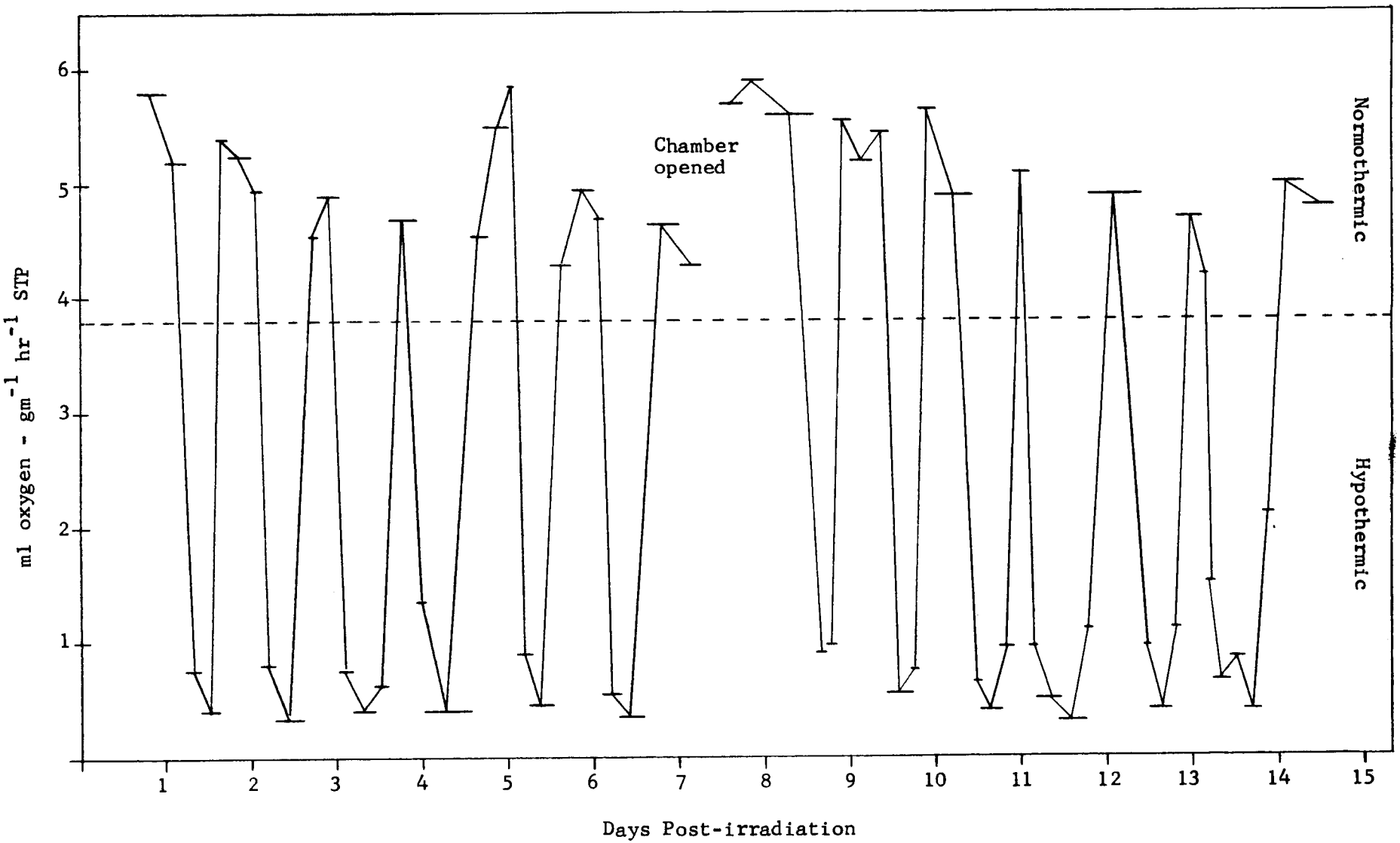

Figure 21. Metabolic activity in P. longimembris (♀) following 1400 r acute whole body cobalt^{60} irradiation (animal at 22°C with food)

noted 1-14 day following irradiation tended to persist.

(5) Rhythms in air as compared to high oxygen atmosphere. In two experiments P. longimembris were kept in air rather than 80 - 90% oxygen: groups (6) and (9) in Table 8.

In item (6), in which six mice were measured as a group, the gradual decline in the level to which arousal occurred indicates that not all of the animals were arousing each day and/or they were not arousing to a normometabolic level. It is probable that some individuals were deeply hypothermic for more than one day at a time; this is usually not observed for animals in 80 - 90% oxygen.

In group (9) in which individual animals were monitored, it is very evident in the data that the mice did not arouse as frequently as those in high oxygen atmosphere, and hence remained in deep hypometabolism for longer periods of time. All mice in this group had been deeply hypothermic for 2.5 to 4 days when the experiment was terminated. It is still possible to see the basis for a 24-hour pattern in the data, since the arousal midpoints are often at multiples of 24-hours when they do not happen to be near 24-hours:

Number	General Pattern	Intervals Between Arousals
one mouse,	consistent 24-hour pattern	mean interval 24.8 (21-28) hrs.
two mice,	24-hour patterns with one instance of 2 x 24-hr pattern	mean interval 24.3 (20-29) hrs. intervals 47, 47.5 hrs.
one mouse,	one instance 24-hour two instances 2 x 24-hour	interval 24.0 hrs. intervals 41, 54 hrs.
one mouse,	one 24-hour interval one instance 3 x 24-hr pattern	interval 25.5 hrs. interval 65.5 hrs.
one mouse,	one instance 3 x 24-hour	interval 73 hour
two mice,	irregular	mean interval 34.5 (30.5-38) hrs.

These intervals collectively give an average interval between peaks of arousal of 23.9 hours, this for 25 24-hour periods or multiples thereof.

In 12 out of 29 instances the arousal from deep hypometabolism was only partial, i.e. not to the normometabolic level. The "responsiveness" of mice in air was decidedly less than those in a high oxygen atmosphere.

Further study is needed to assess the effect of air versus high oxygen atmosphere on the frequency and amplitude of the metabolic rhythm of P. longimembris.

Comparison of metabolic behavior of isolated and grouped pocket mice -

(1) The data suggest that when pocket mice are kept in a group within the same chamber or in a series of chambers on the same air flow there is a synchronization of the metabolic rhythms of individuals.

While there is an obvious group rhythm in grouped individuals (Fig. 18 and 19),

Table 9

Group and Conditions	Arrangement of Animals	Midpoint of Metabolic Lows	Interval Between Metabolic Lows	Incidence of Deep Hypometabolism(6)
(1) 24°C, Food, Day-Night, Dry 80-90% O_2, Sept. 30 - Oct. 4, 1962	7 Mice, Individually housed and monitored	1405 hrs. (1100-1515)	23.9(22.5-25.5)hrs	3/27 or 10.7%
(2) 22°C, Food, Day-Night, Dry 80-90% O_2, April 30 - May 14, 1963	9 Mice, Individually housed and monitored	1148 hrs. (0430-1736)	24.8(13.0-30.0)	35/114 30.7%
(3) 22°C, Starved, Day-Night, Dry 80-90% O_2, May 21 - May 25, 1963	7 Mice, Individually housed and monitored	0800 (0100-1600)	24.5(21.0-28.5)	21/21 100 %
(4) 10°C, Starved, Day-Night, 80-90% O_2 Saturated with water vapor February 19 - February 26, 1963	5 Mice, Individually housed and monitored	0636 (0318-1000)	23.1(20.7-25.2)	30/30 100 %
(5) 10°C, Starved, Day-Night, Dry 80-90% O_2, March 19-March 26, 1963	6 Mice in one chamber monitored together	1033 (0830-1300)	25.2(23.5-28.5)	6/7 85.7%
(6) 20°C, Starved, Dark, Dry Air with 4.5 CO_2, nest material (1) January 4 - January 9, 1963	6 Mice in series of chambers: monitored together	0700 (0245-1325) (1823 (1445-2300))[2]	22.7(19.0-26.8)	100 %
(7) Perognathus inornatus[3] 10°C, Starved, Dark, Air saturated with water vapor, nest material, February 20 - February 26, 1963	6 Mice in series of chambers: monitored together	0640 (0500-0800)	23.2(21.0-25.0)	100 %
(8) 22°C, Food, Day-Night, Dry 80-90% O_2, after 1400 roentgen exposure, March 25-April 8, 1963	9 Mice, Individually housed and monitored	0902 (0718-1018)[4] 1232 (0736-1518)[4]	24.2(20.0-27.0)[4] 23.8(18.0-27.0)[4]	51/96 53.1%
(9) 10°C, No food, Dark, Air Metabolor, heavy animals	8 Mice, Individually housed and monitored	Irregular[5]	23.9(20-73)[5]	85.7%

(1) Nesting material not provided unless specified; animals kept over granulated absorbent, "Drizit."
(2) Midpoints of metabolic highs, which could be read more accurately than lows in this case.
(3) All other data for P. longimembris. These data from Chew (1963).
(4) Values for first and second weeks of two week period. Disturbed at 7 days for weighing.
(5) Irregular; some intervals were multiples of 24 hrs; see text.
(6) Incidence is number of occurrences/ possible theoretical total; possible = number days of experiment.

there is not an equivalent synchronization of individuals that are measured separately at the same time. (This may be a case of social entrainment of rhythms.) (Figure 20)

(2) The data suggest that when animals are kept in groups, there is a much greater tendency for individuals to become deeply hypometabolic and remain so for several days. In figure 18,the group as a whole was hypometabolic from early in the third day onward. Similarly, in figure 19, the group was hypometabolic from the fourth day onward.

It is concluded that the "group" hypometabolism is the result of one or more individuals failing to arouse at the expected time. The "group" rhythm persists because of those animals that do arouse, to create the peaks in the curve.

SUMMARY AND CONCLUSIONS

Metabolic rate, body temperature and related phenomena have been studied in seven species of pocket mice (genus Perognathus), for animals in both normal and hypometabolic states. Most of the data pertain to the Little Pocket Mouse, P. longimembris.

1. Because of its small body size (8-10 gm) P. longimembris intrinsically has a relatively unstable body temperature. Deep body temperature (T_c) varies as much as $5^{\circ}C$ in a day, or $1.5^{\circ}C$ within an hour, when the animal is normometabolic.

2. Within this range of variation, P. longimembris can keep its T_c stable over a range of ambient temperatures, T_a $0^{\circ}C$ to $34^{\circ}C$.

3. Above $T_a = 34^{\circ}C$, T_c rises, in parallel with T_a. P. longimembris can tolerate a T_c of 41.5 to 42.0 at least briefly.

4. At $10^{\circ}C$, fluctuations of T_c clearly depend upon and follow changes in metabolic rate.

5. The temperature gradient from center of body to subcutaneous tissues, $T_c - T_s$, is relatively constant at $3.3^{\circ}C$ from $T_a = 2^{\circ}C$ to $T_a - 14^{\circ}C$. The gradient decreases to about $1^{\circ}C$ at $T_a = 28^{\circ}$ and then remains constant.

There appear to be four zones of temperature regulation, combining physical and metabolic regulations.

6. The average maintenance metabolic rate of normometabolic P. longimembris can be estimated by the formula:

$Y = 12.163 - 0.276X$, where Y is ml $O_{2\ STP}$/gm hr

and X ambient temperature in $^{\circ}C$.

This relationship is valid for animals measured in either air or a high-oxygen atmosphere (80 - 90% O_2).

7. The minimum maintenance metabolic rate attained over a half hour period is about 72% of the average rate.

8. P. longimembris has a range of thermal neutrality from about T_a 33° to 36°C. Within this range, the minimum maintenance rate ($\simeq$ basal rate) is about 1.13 to 1.85 ml/gm hr.

9. This minimum rate is only about half that predicted by metabolic formulae developed for mammals that do not naturally inhabit deserts.

10. Maintenance metabolic rates have been measured for six species of pocket mice (Perognathus flavus, P. longimembris, P. amplus, P. intermedius, P. formosus and P. baileyi), ranging, in this order, from average body weights of 7.5 to 31.0 grams.

11. Over the range T_a 5° - 35°C, these species form a family of curves. Metabolic rates range inversely with body size from Y = 15.007 - 0.405X for P. flavus to Y = 7.272 - 0.185X for P. baileyi.

12. The total conductance (integrating both physical and physiological factors) ranges inversely with body size from 0.40 ml O_2/gm hr for P. flavus, to 0.18 ml O_2/ gm hr for P. baileyi.

13. The lower critical temperature varies from 29.5°C for P. baileyi and P. formosus to 35°C for P. longimembris.

14. P. longimembris deviates somewhat from the family of curves in that its "basal" rate at 35°C and its lower critical temperature are both higher than expected on the basis of body size.

15. For these six species of Perognathus there is a sharp upward increase in metabolic rate at a body size of about 10 grams. A theoretical lower limit to body weight in this genus would seem to occur between 2.5 and 3.5 grams.

16. In the comparisons that were made, the metabolic rates of Perognathus spp. measured in air did not vary significantly from those measured in 80 - 90% oxygen.

17. However, P. longimembris does not show any unusual tolerance for 100% oxygen at one atmosphere. A 50% mortality occurs in four days; dead mice showed obvious pulmonary edema.

18. In states of deep hypometabolism, in air at 10°C, P. longimembris reach minimum metabolic rates of 0.017 to 0.027 ml $O_{2\ STP}$/gm hr. This is in the range of 0.02 - 0.03 found for hibernating mammals.

Individuals in 80 - 90% oxygen had higher minima, 0.09 - 0.22 ml O_2/gm hr.

19. The Q_{10} for the metabolic rate of deeply hypothermic P. longimembris is in the range 1.40 - 2.73; these values are close to the 2.2 for isolated tissues from hibernating mammals.

20. When P. longimembris are deprived of food, almost all individuals become periodically deeply hypometabolic. At T_a 10°C the animals are hypometabolic 60 - 80% of the time; they thus save 67 - 80% of the energy cost of remaining normometabolic.

21. When they are allowed food at 10°C, the incidence of torpidity is low; the median frequency observed was torpidity on 11 to 15 days out of 140 days during January through July.

22. P. longimembris, although normometabolic, stayed in artificial burrows for prolonged periods (up to at least 3 weeks) when T_a was kept at 10°C. This may be their behavior in nature in wintertime.

There is as yet no evidence for prolonged hibernation.

23. Pocket mice (P. longimembris and P. inornatus) demonstrate a circadian metabolic rhythm.

24. This rhythm is obviously in agreement with the natural photoperiod; the mice, which are nocturnally active under natural conditions, show metabolic lows during daylight hours and metabolic highs during nighttime hours. This occurs even when animals are in continuous darkness for 6-7 days.

25. Starvation and low ambient temperatures accentuate the amplitude of the rhythm so that there is an occurrence of deep hypometabolism almost every day.

26. The approximate 24-hour period of the rhythm is not altered by keeping individual pocket mice in continuous darkness, isolating them from sound, exposing them to air with 4.5% CO_2 or to atmospheres saturated with water vapor, or exposing them to 1400 r Co^{60} irradiation.

27. In air, P. longimembris (at 10°C without food) arouse less frequently, i.e. remain deeply hypometabolic for longer periods (up to 2-4 days), but a rhythm of 24-hours or a multiple thereof persists. A high oxygen atmosphere may retard the development of periods of sustained hypometabolism.

28. When starved mice are kept in groups, there is a tendency for their rhythms to become synchronized, and also for some individuals to "drop out of the rhythm" for one or more days by remaining deeply hypometabolic.

The present data are adequate for predicting the logistics requirements of Perognathus longimembris for space biopack experiments.

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UNPUBLISHED PRELIMINARY DATA

RESPONSE OF THE POCKET MOUSE TO IONIZING RADIATION

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INTRODUCTION

Pocket mice (Genus: Perognathus) have been proposed as particularly suitable subject matter for space biology research by virtue of their unusual physiology which in turn allows significant compromises in the life support requirement for mammals in biosatellites. The resultant savings in payload weight coupled with the small size of the animals were anticipated to permit experimental designs with statistically significant numbers of animals and good reliability.

Since there is a dearth of physiological data on the pocket mouse, baseline data points had to be established prior to designing satellite experiments. Among the baseline studies undertaken was the response of the pocket mouse to ionizing radiation.

It was anticipated that its $LD_{50/30}$ would be comparable to that of other small mammals as was found for the closely related kangaroo rat (8). In the course of these studies, however, the pocket mouse was found to demonstrate a high degree of radiation resistance. In this respect it differs greatly, not only from its close relatives, but from all other mammals.

MATERIALS AND METHODS

Pocket mice are heteromyid rodents indigenous to the arid regions of Western United States and parts of Mexico. Taxonomically, Dipodomys (kangaroo rat), Microdipodops (kangaroo mouse), and Perognathus (pocket mouse) are grouped in the subfamily Perognathinae (Heteromyidae). This grouping not only reflects morphological similarities, but also ecological and physiological ones. For example, the ability to subsist on air dry seeds with no requirement for drinking water or succulent foods appears to be a physiological characteristic the three genera have in common (3).

The capability of becoming hypothermic under certain adverse environmental conditions also occurs among the heteromyids. Lack of food and low environmental temperature is the trigger for this phenomenon, which has been documented in species of Perognathus and Microdipodops (1,2). There are no reports of naturally occurring hypothermia in the genus Dipodomys, although it can be induced.

The genus Perognathus includes 26 species. Perognathus longimembris, weighing approximately 8.5 grams, is among the smallest of the genus; and is, indeed, one of the smallest mammals. P. formosus weighing approximately 20 grams compares favorably in size to the common laboratory mouse. Pocket mice are available in large numbers, easily live-trapped, and tractable when brought into the laboratory. Their small size, ability to exist on dry food, and concentrated body waste make them particularly easy to maintain.

Animals were selected according to sex and weight from a large collection of Perognathus which were live trapped in the field and maintained in our laboratory. From field data and available information on population dynamics of the pocket mouse, it is assumed that approximately 80% of the animals used in this study were just under one year old at the time of irradiation. The others were just under two years old.

SURVIVAL STUDIES - X-RAYS

One hundred and twenty-five (125) healthy adult ♂ P. longimembris with a mean weight of 8.7 gms (range: 7.4 - 10.5 gms) were segregated from the main colony and divided into five groups of 25 each (Table 1). Group assignments were made from a table of random numbers (animals are numbered consecutively as they arrive from the field). In this manner groups are randomized as to age and collection site of the mice.

Four groups received single exposure whole body irradiation at doses of 400, 600, 800, and 1000 r delivered from a 250 KVP 15 ma X-ray machine*(0.47 mm Cu inherent, 65.0 cm TOD, and 20.52 r/m dose rate). A control group was handled similarly to the irradiated groups except for the actual irradiation.

Animals were transported to and from the radiation site, a round trip of approximately 20 miles, and irradiated in compartmentized plastic boxes. The animals were

* Use of the radiation facilities at the Department of Nuclear Medicine and Biophysic Biophysics, Laboratory of Nuclear Medicine and Radiobiology at the University of California at Los Angeles is gratefully acknowledged.

Table 1. Experimental Design

X-Ray Survival

Dose (r)	Species	Sex	Number
0	P. longimembris	♂	25
400	"	"	"
600	"	"	"
800	"	"	"
1000	"	"	"

Co^{60} Survival

0	P. longimembris	♂	25
0*	"	"	"
800	"	♀	"
1200	"	♂	"
1400	"	"	"
1600	"	"	"
2000	"	"	"
0	P. formosus	♂ & ♀	9
0*	"	"	24
600	"	"	10
1200	"	"	10
1400	"	"	23
1600	"	"	24
1800	"	"	9

Hematological Studies

0	P. longimembris	♂	25
0	"	♀	49
0	"	"	25
400	"	♂	25
400	"	♀	25
1400	"	♂	10
1400	"	♀	50

* Control groups are duplicated because radiations were performed on three different dates.

in these boxes for a time period of approxmately four hours. Although their movement was limited, ventilation was adequate as judged by their apparent comfort. Because of the unusual amount of handling required in getting the animals to the radiation source, a group of 25 White Swiss mice were also subjected to 600 r whole body irradiation at the same time and in the same manner as were the pocket mice. In addition, a control group of White Swiss mice were retained.

During irradiation, the boxes were placed on a rotating device which insured a uniform distribution of radiation over 99% of the field. Animals were oriented in the compartments in such a manner that irradiation was received dorsoventrally.

After irradiation the animals were returned to our laboratory and restored to their original caging. All pocket mice in our laboratory are maintained in individual gallon-size, wide-mouth jars which contain two to three inches of sand. A mixture of grass seed ("parakeet" seed), rolled oats, and sunflower seed is made available ad libitum. No drinking water is required.

Temperature control in the laboratory is set at 22° C. Normally, at night it is maintained between 20° and 22° C. During the day it varies between 22° and 24° C, with occasional excursions to 25° C. Relative humidity is maintained at 50 ± 5%.

During the first month following irradiation, animals were checked twice daily. Dead animals were autopsied as soon after death as possible.

SURVIVAL STUDIES Co^{60}-RADIATION

(a) P. longimembris

Seven groups of 25 adult male P. longimembris were established as described for the X-ray studies. Mean weight of the animals used was 8.3 gm (range: 6.0 - 11.9 gm). Radiation doses of 1200, 1400, 1600, 1800, 2000 r were delivered from a 10,000 curie Co^{60} source at a dose rate of 102 r/m.

One group of 25 adult female P. longimembris was administered 800 r from the same source and in the same manner. Mean weight of females was 8.5 gms (range: 6.8 - 10.2 gms).

Appropriate control groups were retained.

All other techniques and conditions for these studies were as described above for the X-ray studies.

(b) P. formosus

Thirty-eight (38) adult P. formosus of both sexes were divided into four groups of 9 or 10 each and subjected to 600, 1200, and 1800 r Co^{60} radiation. One group

was retained as a control group. The mean weight of these animals was 18.7 gms (range: 15.5 - 21.7 gms).

In a second radiation series, seventy-one (71) P. formosus of both sexes were divided into three groups of 23 or 24 each. One group subjected to 1400 r, another to 1600 r Co^{60} irradiation, and a third group was retained as controls. The mean weight of these animals was 20.4 gms (range: 14.0 - 28.1 gms). Radiation was delivered and techniques used were as described for P. longimembris.

HEMATOLOGICAL STUDIES

Fifty (50) adult male P. longimembris with a mean weight of 8.8 gms (range 7.7 - 10.9 gms) were segregated from the main colony and divided into two groups in the same manner as described in the X-ray studies.

One group of 25 animals received 400 r whole body X-irradiation. A group of 25 controls were handled identically to the irradiated except for the actual irradiation.

On the 1st, 3rd, 5th, 7th, and 9th days, post-irradiation blood samples were taken from five irradiated and five control animals. Different animals were used on each of the days so that each animal was bled just once. Peripheral blood samples were obtained by the method of tail transection. Total erythrocytes, leucocytes, and differential counts were made and microhematocrits were obtained.

In a second blood series, four groups of adult female P. longimembris were established. One group of 25 animals received 400 r; a second group containing 50 animals received 1400 r Co^{60} radiation. A group of 50 manipulation controls and 25 laboratory controls were retained. The mean weight of these animals was 8.1 gms (range: 6.5 - 11.3 gms). Total erythrocyte, leucocyte, and platelets counts, differentials, hemoglobins, and hematocrits were obtained over the 10-day period immediately following irradiation.

Sufficient numbers of animals were irradiated or used as controls in each group so that no animal was bled more than once. Daily blood samples of five manipulation controls over the 10-day period were obtained. Ten 1400 r animals were bled on alternate days during the period: 1st, 3rd, 5th, 7th, and 9th. Five 400 r and five laboratory control animals were bled on the 2nd, 4th, 6th, 8th, and 10th days.

Total erythrocyte and leucocyte counts, differentials and microhematocrits were obtained throughout the period. Hemoglobin determinations were made on the 1st and 2nd days and on the 9th and 10th days post-irradiation. Platelet counts were made on several control animals and a small number of irradiated animals on the 9th and 10th days.

The Coulter electronic counter was used for total counts. Phase-microscopy was used for direct platelet counts. Hemoglobins were determined by the acid-hematin method. Slides were stained with Wright Stain for differential counts.

RESULTS

Probability plots for acute dose-mortality data are shown in figure 1. The $LD_{50/30}$ values obtained for P. longimembris is 1510 r; that for P. formosus is 1280 r. In figure 1 mortality curves for these two species are compared with that for female CF_1 mice from the data of Patt et al. The $LD_{50/30}$ for CF_1 mice is 628 r. The ST_{50} for the White Swiss mice irradiated at 600 r in this experiment was 19 days.

Acute mortality curves for Perognathus species had the typical sigmoid shape exhibited by dose-mortality data for other mammals. There were no deaths prior to six days and, as shown in figure 1, significant numbers of deaths occurred in high dose groups only. That is, it was necessary to administer doses in excess of 1200 r to obtain significant early deaths in P. formosus. Similarly doses in excess of 1400 r were necessary to obtain significant numbers of deaths in P. longimembris.

Continued observations on survival for periods up to 29 weeks post-irradiation indicate only a gradual decline in survival in any of the radiation groups. Animals irradiated at 1000 r, for example, have 80% remaining at 29 weeks; those receiving 1400 r have 75% remaining at 21 weeks.

Gross autopsy findings of those animals that died during the acute period reveals that deaths were probably due to either acute respiratory disease, gastro-intestinal damage, or hemorrhages. Internal bleeding, either gastrointestinal or intra-cranial, was observed in approximately 70% of the dead animals. Complete autopsy results will be the subject of a subsequent report when all of the irradiated and control animals are dead.

Tables 2-5 present various blood values of irradiated and control pocket mice. Inspection of erythrocyte data suggest that no marked depression of erythropoiesis occurred during the early post-irradiation period. Slight reductions in mean values of erythrocyte counts, hematocrits, and hemoglobins are seen in animals which were administered 1400 r. These reductions are not statistically significant even at nine days post-irradiation. They suggest, however, that at the 1400 r dose level the pocket mouse may demonstrate a typical radiation anemia at approximately the same time that it occurs in other mammals following just sublethal irradiation. Stippled and nucleated erythrocytes were observed on blood slides of irradiated animals as early as the first day post-irradiation.

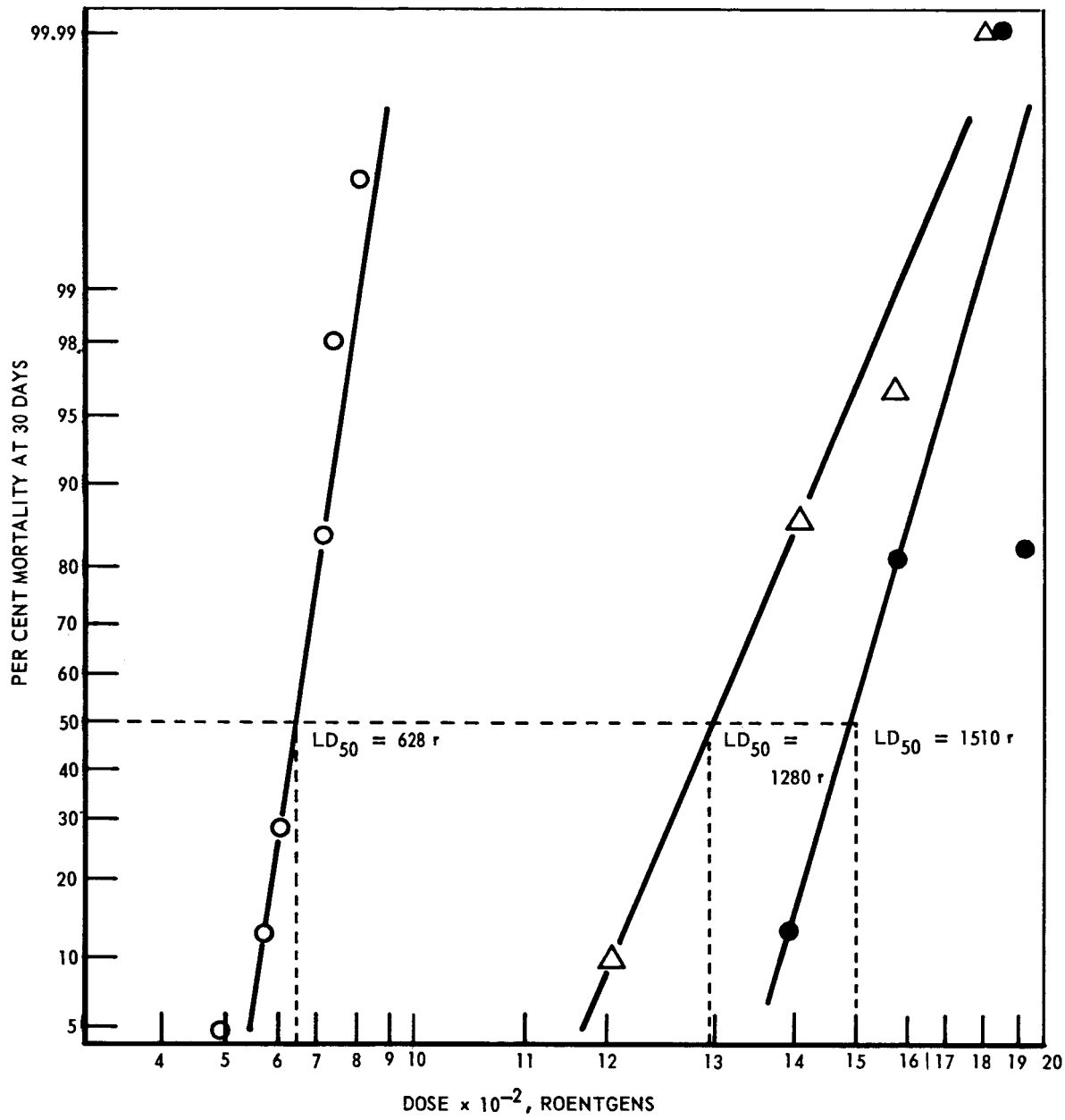

FIGURE 1 ACUTE MORTALITY OF MICE EXPOSED TO VARYING DOSES OF IONIZING RADIATION

(ABSCISSA, LOG_{10} SCALE; ORDINATE, PROBABILITY SCALE. OPEN CIRCLES – ♀ CF_1 MICE (DATA OF PATT ET AL, 1953); TRIANGLES – PEROGNATHUS FORMOSUS; FILLED CIRCLES – PEROGNATHUS LONGIMEMBRIS.)

	Day	Erythrocytes* per cu mm		Leucocytes** per cu mm		Lymphocytes %		Hematocrit %	
		$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	range
400 r total body X-irradiation	1	14.0	± 1.7	6.4	± 2.1	55	± 16	55	(50-59)
	3	14.1	± 1.4	5.5	± 2.8	55	± 21	53	(50-57)
	5	12.5	± 2.7	5.1	± 1.6	89	± 8	52	(50-52)
	7	13.4	± 1.6	4.7	± 1.0	75	± 25	53	(52-54)
	9	12.5	± 2.8	8.3	± 2.9	65	± 28	57	(52-56)
Non-irradiated controls	1	13.6	± 1.7	7.5	± 4.7	76	± 8	52	(49-55)
	3	14.2	± 0.7	8.8	± 2.2	74	± 13	53	(50-56)
	5	13.8	± 0.9	11.4	± 2.3	83	± 5	53	(47-59)
	7	14.3	± 1.7	9.1	± 3.0	70	± 16	56	(54-58)
	9	13.6	± 1.6	10.3	± 3.2	78	± 16	56	(53-58)

* $X10^{-6}$

** $X10^{-3}$

Table 2. Blood Values of 25 Adult Male Perognathus longimembris and 25 Controls Sampled in Groups of 5 on Days 1, 3, 5, 7, and 9 Following Sublethal Total Body X-Irradiation.

E R Y T H R O C Y T E S *

Day Post-Irradiation	400 r		1400 r		Manipulation Control		Laboratory Control	
	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ
1			14.0	2.5	14.8	1.7		
2	13.8	1.9			14.5	0.4	13.4	1.8
3			12.3	1.6	13.0	0.5		
4	12.8	1.0			13.1	0.8	13.4	1.8
5			12.7	0.8	12.5	1.1		
6	12.1	0.7			11.5	1.0	12.0	1.0
7			13.2	1.5	13.3	0.7		
8	15.9	2.3			13.8	1.7	15.5	2.2
9			11.5	2.7	13.8	0.3		
10	12.6	1.6			13.6	1.1	13.1	0.5

L E U C O C Y T E S * *

1			3.9	1.9	6.6	4.5		
2	6.9	2.1			9.6	3.3	11.4	5.9
3			3.0	1.3	8.1	4.1		
4	2.6	1.1			8.4	3.9	8.8	6.5
5			1.6	0.8	9.3	4.9		
6	5.3	3.8			6.8	2.2	7.0	1.7
7			1.5	0.9	7.5	2.0		
8	5.9	2.2			7.3	2.0	6.9	2.6
9			0.9	0.7	6.0	3.6		
10	7.0	5.3			5.4	2.5	6.6	3.7

* Cells per cu mm $X10^{-6}$

** Cells per cu mm $X10^{-3}$

Table 3. Total Blood Cell Counts of Pocket Mice (Perognathus longimembris) serially Sampled During the 10-day Period Immediately Following Total Body Co^{60} Irradiation.

Day Post-Irradiation	% L Y M P H O C Y T E S							
	400 r		1400 R		Manipulation Control		Laboratory Control	
	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range	$\bar{X}$	range
1			46	(20-87)	75	(40-95)		
2	56	(22-95)			92	(88-95)	83	(64-94)
3			39	(12-81)	70	(68-85)		
4	64	(59-73)			83	(77-92)	85	(78-92)
5			96	(90-100)	79	(72-91)		
6	73	(68-81)			85	(78-92)	87	(81-91)
7			90	(60-100)	77	(65-88)		
8	70	(40-92)			73	(63-88)	73	(46-84)
9			68	(42-100)	77	(48-95)		
10	69	(60-76)			68	(58-75)	69	(58-79)

Table 4. Percent Lymphocytes of Pocket Mice(Perognathus longimembris) Serially Sampled During the 10-day Period Immediately Following Total Body Co^{60} Irradiation.

HEMATOCRIT %

Day Post-Irradiation	400 r			1400 r			Manipulation Control			Laboratory Control		
	$\bar{X}$	Range	No.	$\bar{X}$	Range	No.	$\bar{X}$	Range	No.	$\bar{X}$	Range	No.
1				56	50-61	2	57	54-60	2			
2	54	50-56	4				58	53-61	5	53	49-58	5
3				51	46-58	15	53	52-55	4			
4	49	45-54	5				57	55-60	5	53	49-58	5
5				52	46-54	8	55	47-61	5			
6	53	50-56	5				55	53-56	5	53	50-56	5
7				52	46-58	13	55	53-58	4			
8	56	51-60	4				51	49-54	4	56	51-60	4
9				46	22-56	9	57	54-62	4			
10	55	51-58	4				55	52-57	5	55	51-58	4

HEMOGLOBIN %

1				17.9	15.9-20.4	4	16.9	16.1-18.4	3			
2	16.2	15.2-17.3	5				17.2	15.3-18.8	5	15.9	14.9-16.8	5
9				13.8	7.1-16.0	6	17.2	16.9-17.6	4			
10	16.2	13.8-17.7	3				17.6	16.0-18.7	5	17.9	16.5-18.9	5

PLATELETS*

9				20.4	0-97.8	5	59.0	43.5-73.0	3			
10	70.5	---	1				64.1	30.0-98.2	2	83.4	59.8-99.3	4

*Values per cu mm $X10^{-4}$

Table 5. Selected Blood Values of Pocket Mice (Perognathus longimembris) Sampled During the 10-day Period Immediately Following Total Body Co^{60} Irradiation.

As is typical of mammals, leucocytes of the pocket mouse respond to irradiation within 24 to 36 hours. This early response is manifested as a general leucopenia (figure 2). Since total leucocyte counts are normally extremely variable, it seems reasonable to present the data as in figure 2, comparing counts of irradiated animals with a range of values (the stippled area) which represents the normal variability in control values. Mean values for 400 r animals fall below the normal control range on the 3rd and 4th days only. Mean values for 1400 r animals fall below the normal control range on every day tested.

Differential counts reveal that the leucopenia reflects an initial lymphopenia followed by a marked but transient neutropenia at both the 400 r and the 1400 r dose levels.

Differential counts also indicate that the lymphopenia is a result of the prompt disappearance of small lymphocytes following irradiation. Large lymphocytes outnumber small lymphocytes in blood smears on the 1st through the 7th day following 1400 r irradiation. Large lymphocytes predominate on the 4th, 6th and 8th days following 400 r irradiation, but not on the 2nd and 10th days. On a whole, small lymphocytes were found in greater numbers than large lymphocytes in blood smears of controls.

Platelets were markedly depressed in four of five 1400 r animals. Four of these animals had counts ranging from 0 to 25,000 per cu mm on the 9th day post-irradiation: one had a normal count (978,000 per cu mm). Platelet counts of nine control animals ranged between 300,000 and 993,000 per cu mm. The one 400 r animal whose platelets were counted had a value of 705,000 per cu mm at ten days post-irradiation.

DISCUSSION

Blood responses of the pocket mouse to total body irradiation follow the same general pattern as seen in other mammals (9). It is necessary, however, to administer to Perognathus much larger radiation doses to produce changes of comparable magnitude. For example, hematological responses of Perognathus administered 400 r total irradiation resemble those of the kangaroo rat which was administered 50 r (8). Perognathus administered 1400 r demonstrates hematological changes comparable to other mammals receiving 400-500 r total body irradiation (5).

Depending upon dose, maximum leucocyte depression occurs between four and nine days following irradiation. Differential counts indicate that leucocyte changes reflect an initial lymphopenia which is followed in a few days by a marked reduction in peripheral neutrophils. This course is normal for small mammals following sublethal irradiation (6).

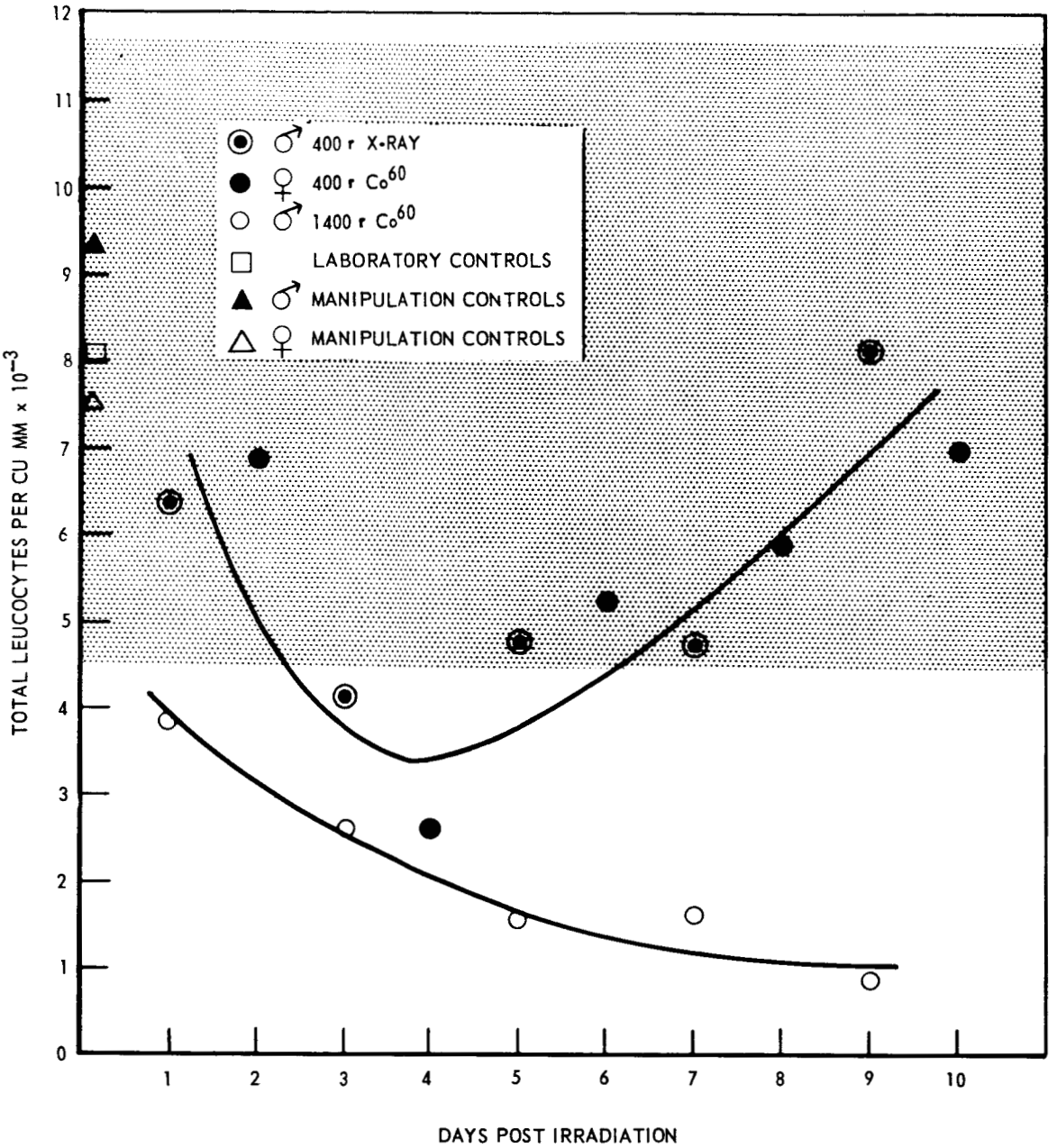

FIGURE 2 EARLY HEMATOLOGICAL CHANGES IN PERIPHERAL BLOOD OF PEROGNATHUS LONGIMEMBRIS FOLLOWING TOTAL BODY IRRADIATION

(SHADED AREA REPRESENTS ±1 STANDARD DEVIATION OF LABORATORY CONTROL MEAN. CURVES FITTED BY INSPECTION.)

Perognathus survives doses of acute radiation which are significantly higher than those survived by other mammals, when the irradiation is delivered under comparable conditions. The ST_{50} reported for Dipodomys, for example, is 10.3 days at a delivered dose of 550 r (8). In P. formosus and P. longimembris 1600 r and 1800 r, respectively, are required to obtain approximately the same ST_{50}.

Since Perognathus has the capability of going hypothermic, a comparison might logically be made between it and other small mammalian hibernators. It is well documented that the state of hibernation confers protection against the lethal effects of radiation (10). On the other hand, mortality of hibernators when irradiated while in a normothermic condition follows closely that of any other mammal (10). In other words, the ability to hibernate does not appear to confer a special ability to resist the effects of radiation, if the irradiation is delivered while the animal is normothermic.

In contrast to other hibernators, the radioresistance noted in Perognathus occurs when the animal is irradiated in a normothermic state. There is insufficient evidence to conclude at this time that the resistance is either related or unrelated to the ability of Perognathus to hibernate.

Reported $LD_{50/30}$ doses among the mammals range from a few hundred to several hundred roentgens, when the irradiation is delivered as a single acute dose and at a comparable rate (4). Variation in $LD_{50/30}$ doses among the mammals probably reflect a number of real and experimentally produced differences between the species investigated. Despite the wide differences in sensitivity certain basic mechanisms of death are quite similar. At doses less than 1000 r, death is caused by hematopoietic failure and occurs within the first three or four weeks, but not earlier than the 2nd week following irradiation. At doses greater than 1000 r, death is the result of irreparable damage to the gastrointestinal epithelium and occurs within the first week or two following irradiation. Much higher dose levels (5,000-10,000 r) produce extremely early "CNS" death. $LD_{50/30}$ doses are not derivable from such high doses.

The significance of $LD_{50/30}$ doses reported for Perognathus is the fact that they survive doses (1000 r) which are uniformly fatal to other mammals via the gastrointestinal syndrome. Secondly, they survive doses that even if by some life-saving treatment other mammals are protected from the gastrointestinal death, hematopoietic failure is certain to ensue and ultimately cause death. Methods of protecting against hematopoietic death are known but are not significantly successful if applied following high dose irradiation.

The two to threefold increase of $LD_{50/30}$ values for Perognathus over those for other mammals suggests that both hematopoietic and gastrointestinal damage is

ameliorated. Hypoxic hypoxia produces the same protection in other mammals. Dehydration is also protective (7). Post-irradiation hypothermia reduces irradiation damage. These agents are mentioned from the long list that is known to alter radiation response, because there is some evidence that one or all of these may be acting in Perognathus. Follow-up work in this laboratory is designed to elucidate the mechanism of radioresistance in Perognathus.

SUMMARY

abstract 21523

1. Several hundred pocket mice representing two species, Perognathus longimembris and P. formosus, were administered single whole-body X- and gamma-radiation. Exposure doses of 400, 600, 800, 1000, 1200, 1400, 1600, 1800, and 2000 r were used.

2. Radiation at the highest dose levels only (>1200 r) resulted in significant numbers of acute (30-day) deaths. The $LD_{50/30}$ for pocket mice in this experiment (1280 r P. formosus: 1520 r P. longimembris) is two to three times that reported for related mammalian forms.

3. Post-irradiation hematological changes as evidenced by total and differential blood counts, hematocrits, hemoglobins, etc., corroborate the extreme radiation insensitivity reflected in survival curves. Leucocyte depression is maximum at five days post-irradiation. At 1400 r the magnitude of the leucocyte depression is of the same order as that observed in other rodents receiving 400 - 500 r.

4. Since radiation was administered while the animals were in a normothermic state, the low mortality suggests a high degree of natural radiation resistance. This resistance may be related to certain unique physiological adaptations of the pocket mouse which enable it to survive in the desert environment.

5. In contrast, the kangaroo rat, which is a desert form with similar adaptations, has an LD_{50} which corresponds closely to that of other small mammals.

6. These results suggest that Perognathus may be the most radio-resistant of any mammal tested to date.

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METABOLIC RATES OF IRRADIATED PEROGNATHUS LONGIMEMBRIS

P. Hayden and J. J. Gambino

INTRODUCTION

There is evidence that slowing an animal's "rate of living" following lethal total body irradiation may delay the onset of radiation damage. For example, survival time of frogs was greatly increased by keeping them at 5^{o} to 6^{o} continuously after lethal exposure (1). In mammals, post-irradiation hypothermia or hibernation postpones the manifestation of radiation effects (2).

It follows that when 30 day survival is used to judge radiation sensitivity erroneous conclusions might be drawn, if by one means or another a mammal "turned down its thermostat" in the period following exposure. In view of the fact that Perognathus can normally undergo periods of hypothermia, it appeared judicious to investigate post-irradiation metabolic rate in this extremely radiation resistant genus.

MATERIALS AND METHODS

Twelve adult Perognathus longimembris (11♂, 1 ♀) were administered a 1400r total body dose of Co^{60} radiation. The conditions of radiation and a description of the animals is reported in the preceding paper. The 1400r dose was chosen because few acute deaths normally result from this dose level in P. longimembris. All 12 animals survived the radiation. Approximately 4 hours after being irradiated 8 of the animals were placed in the metabolor for an initial oxygen consumption study period of two weeks. The metabolor is described in the section of this report dealing with metabolic studies. Conditions of the experiment were: $T_A = 22^{o}C$; high oxygen concentration(80-90%); low relative humidity (<10%), normal photoperiod, excess food.

The group was again placed in the metabolor at 79 days post-irradiation. The duration of this second oxygen consumption study was 4 days, otherwise all conditions were the same as in the first.

In addition, at 50 days post-irradiation, these same animals were used in an experiment to determine the relationships of metabolic activity to ambient temperature. This group of animals was exposed to ambient temperatures varying from $5^{o}C$ to $35^{o}C$.

Three animals died during the first oxygen consumption experiment. Two others died subsequently. The seven survivors of the 12 irradiated animals exhibit greying, otherwise appear normal and vigorous at 5 months post irradiation.

RESULTS

During the post-irradiation periods tested, oxygen consumption data suggest that the metabolic level of sublethally irradiated pocket mice, when maintaining normal body temperature, is within the normal limits established for these mice at 22°C (Figure 1).

During the first two-weeks study a diurnal rhythm ranging from normal metabolism to torpor was evidenced. This rhythm was pronounced in 4 of the 8 animals. An extreme example of this phenomenon is shown in figure 21*. In general, the periodic torpor was accentuated during the second week.

There was an overall total weight loss of 10% during the first week, 5% of which was regained during the second week. The total weight loss during the entire period was less than 6%.

Of the 4 animals that showed a well defined diurnal torpor during the first 2 week study, only 2 had any indication of a timed sequence when tested again at 79-83 days post irradiation. The weight loss of those that showed torpor was 5.5% compared to a weight loss of 3.0% for those that showed no torpor.

When this group of animals was exposed to ambient temperatures varying from 5° to 35°C (50 days post-irradiation), there was an indication of deviation from the normal oxygen consumption curve at the lower temperatures (Figure 2). The increase is approximately 17% at 0°C.

DISCUSSION

Results of this limited study suggests that post-irradiation hypothermia cannot account for the extreme radiation resistance previously observed in Perognathus (see preceding section). The diurnal periods of torpidity are not exhibited by all the irradiated animals and are not considered of sufficient duration to reduce the overall "rate of living" during the 30 days post-irradiation period. The reduced metabolic level during this period is judged insufficient to cause a significant delay in acute deaths.

* In part 1 Metabolic Studies

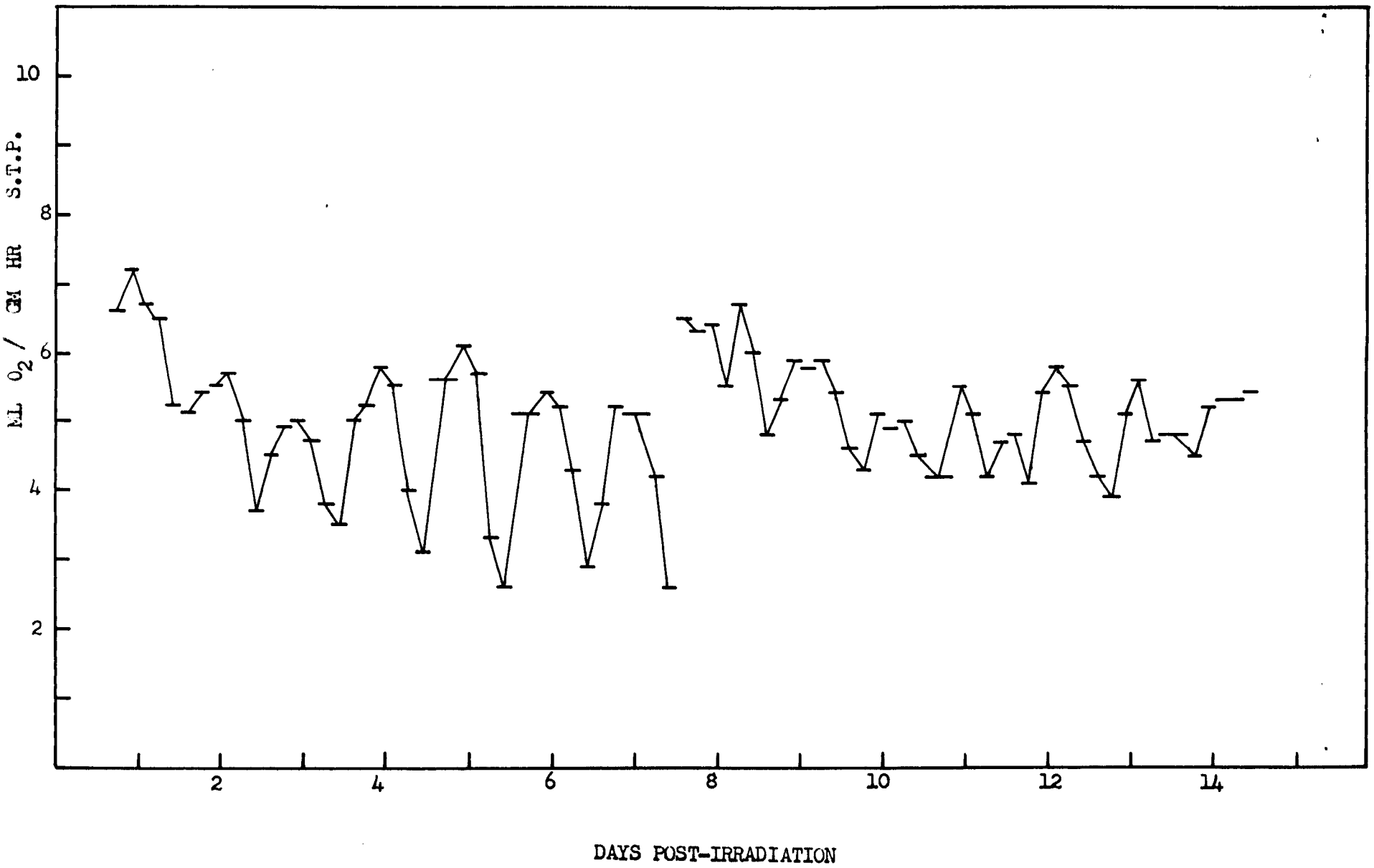

Figure 1. Integrated oxygen consumption of 8 individually monitored P. longimembris following exposure to 1400 r Co^{60} radiation. Maintained at 22 °C with food.

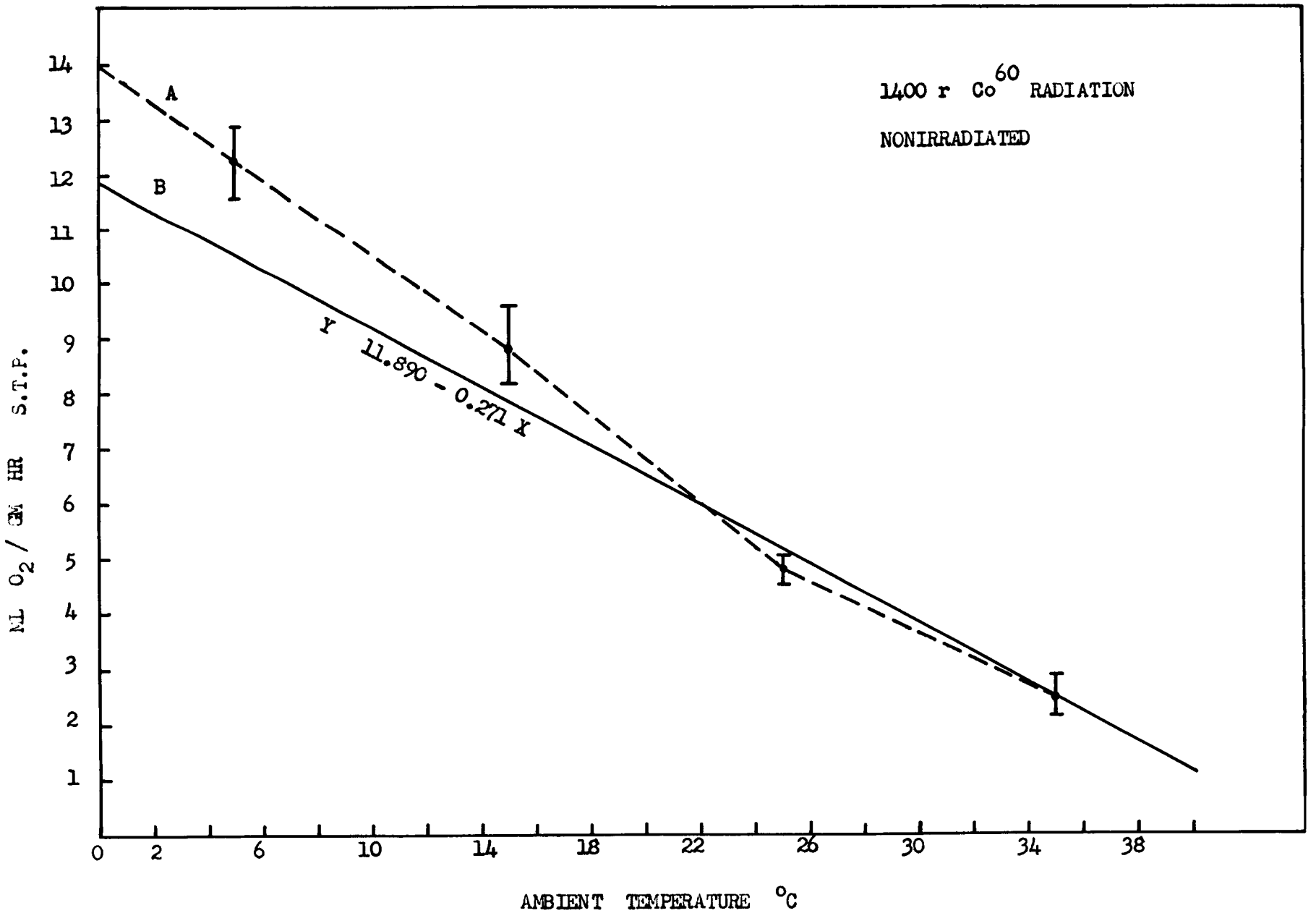

Figure 2. Maintenance metabolic rate of normal normothermic P. longimembris (A) compared with that of P. longimembris (B) which received 1400 r total body irradiation 7 weeks prior to date of experiment. (Verticle lines show 1 SD)

It is possible that the diurnal torpidity reported here is the same kind of cycle documented in P. californicus (3) and also noted in this laboratory in fasting Perognathus. In P. californicus it was determined that a reduction in food supply was sufficient for the animal to respond with torpor as a method of energy conservation (3).

Although the irradiated pocket mice had food available at all times, it is possible that they were anorexic and were responding to a "less-food" situation.

Weight loss data may corroborate this hypothesis. The animals apparently were "off-feed" during the first week post-irradiation. It is possible that the overall weight loss recorded for the 2-week period represents water loss in the dry atmosphere of the metabolor.

The increased metabolic rate at temperatures below 22°C of the animals exposed to a wide range of ambient temperatures may be explained by the direct effects of ionizing radiation on pelage. Post-irradiation epilation had occurred in most of the animals with the normal hair being replaced by nearly white hair. By inspection it appears that the soft underfur is missing or incomplete in this replacement coat. In effect the conductance of the animal has been increased (insulation decreased). In order for the animal to maintain a normothermic condition, the rate of metabolism must be increased to "make up" for the loss of insulation.

SUMMARY

1. The metabolic level of the mice when maintaining normal body temperature was within normal limits.

2. A pronounced diurnal torpor was observed in several irradiated pocket mice. This appears to be a response to a "less-food" situation of possible anorexia of the irradiated mouse.

3. The diurnal torpor was not as strongly evidenced approximately three months post-irradiation.

4. Deviation from the normal metabolic-ambient temperature relationship is believed to be associated with an increase in conductance with the post-epilation pelage.

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Northrop Spruce Lake

NASA-91

UNPUBLISHED PRELIMINARY DATA

PART III

GENERAL BIOLOGY

BLOOD VALUES OF POCKET MICE (PEROGNATHUS)

J. J. Gambino and N. K. Miyahara

INTRODUCTION

Measurements of blood values provide some of the most accessible and reliable means of assessing the physiological state of an animal. Alterations in the blood picture may result from or reflect such diverse phenomena as normal cyclic metabolic patterns as seen in diurnal and seasonal rhythms or in hibernation and estivation; reaction to both physical and psychological stressing agents; and many kinds of pathological conditions.

Because knowledge of the hematological picture is so basic to the understanding of the many facets of an animal's physiology and behavior, the present study of Perognathus blood was undertaken as a prelude to physiological experiments with this genus. Baseline values of Perognathus blood obtained in this study have been used to assess the effects of exposure to ionizing radiation (see section on radiobiology). They may also be used to complement other programs in this laboratory in which metabolic rhythms, hypothermia, and similar phenomena are being investigated.

MATERIALS AND METHODS

Animals:

The animals used in this study were sampled at random from a large collection of Perognathus which were live trapped over a two year period and maintained in our laboratory. From field data and available information on population dynamics, it is assumed that approximately 80% of the animals used in this study were just under one year old. The others were just under two years old.

Pocket mice are heteromyid rodents indigenous to the arid regions of western United States and parts of Mexico. Taxonomically, Dipodomys (kangaroo rat), Microdipodops (kangaroo mouse), and Perognathus (pocket mouse) are grouped in the subfamily Perognathinae (Heteromyidae). This grouping not only reflects morphological similarities, but also ecological and physiological ones. For example, the ability to subsist on air dry seeds with no requirement for drinking water or succulent foods appears to be a physiological characteristic the three genera have in common (1).

The genus Perognathus includes 26 species. Perognathus longimembris, one of the two species used in this study, weighs approximately 8.5 grams. P. formosus, the other species studied, weighs approximately 20 grams.

Animals are maintained in our laboratory in individual gallon-sized, wide-mouth jars containing 2-3 inches of clear sand. The animals are allowed free access to a mixture of grass seed, rolled oats, and sunflower seed. No drinking water is required.

Temperature is maintained at 22°C ± 2°C and relative humidity is maintained at 50 ± 5%. Both male and female mice were used in this study as noted.

Method of bleeding:

The single constraint on the technique of obtaining blood was that it did not necessitate sacrificing the animal. Many techniques of bleeding small animals routinely are described; i.e., jugular vein, heart puncture, cul de sac, orbital sinus, tail incision, and tail transection. During the course of this study each of these techniques was attempted on the pocket mouse. However, the small size of this animal precluded all but the tail transection technique for routine success. Even the tail transection technique had to be used in conjunction with a tail warming method.

The following method of collecting blood adapted from the UFAW Handbook (8) was used:

1. The animal is lightly anaesthetized with ether.
2. The animal is wrapped in a small strip of cloth towelling to maintain warmth and partially restrain it.
3. The animal, still wrapped in the towelling, is inserted into a plastic tube approximately 1-1/2 inches in diameter and 3 inches long.
4. The nose of the animal is allowed to protrude through a hole bored in the blunt end of the tube.
5. The tail is extended through a grooved rubber stopper inserted in the open end of the tube.
6. The tail is immersed in warm water (45°C) for a period of 2 minutes.
7. Tail is removed from the water, wiped several times with 95 percent alcohol, then with xylene; finally it is dried.
8. A small portion of the tail (approximately 3 or 4 mm) is excised using a sharp scalpel.

As the blood appeared at the cut end of the tail, it was collected as follows:

1. Five lambda in a micropipet for red and white cell counts.
2. ~10-15 lambda in a microhematocrit tube for hematocrit determination.
3. Several drops for smears on standard slides for differentials.
4. 0.010 cmm in a hemoglobin pipette for hemoglobin determination.
5. 25 lambda in blood diluting pipette for platelet counts.

Methods of making measurements:

Total red and white cell counts were performed using the Coulter Electronic Counter (Model A). Since blood sample size was so small, the standard Coulter counting procedure had to be modified. The following method of dilutions has been proved reliable:

1. First dilution: 5 lambda of blood is added to 10 ml of physiologic saline.
2. Second dilution: .1 ml of the diluted sample is added to 10 ml of physiologic saline.
3. Erythrocyte counts are made on the second dilution (1:100,000).
4. 0.1 ml of 1 per cent Saponin is added to the first dilution and after a 15-minute time lapse the sample is counted.

Reproducible results are obtained using this method as long as normal care is exercised in pipetting techniques and certain precautions specific to the use of the Coulter Counter are taken. These precautions include filtration of the physiologic saline and the Saponin solution through Whatman No. 2.

The standard microhematocrit technique is very successful as long as a sufficient supply of blood is available. Blood is drawn up into a microhematocrit tube by capillary action. A plastic sealing cap or plastiseal is used to seal the tube. The blood is spun for five minutes in a Micro-Capillary Centrifuge (International, Model M.B.) and the hematocrit is read on a logarithmic spiral type reader.

Total erythrocyte counts obtained concomitantly with hematocrits provide a means of determining average volume of a single red cell or mean corpuscular volume (MCV).

Blood smears are made using the standard method of placing a drop of blood on a clean glass microscope slide and spreading it with the edge of a second slide. Some difficulty in obtaining an even distribution of cells on the slides is experienced because of the rapid clotting time of the blood.

Blood smears are stained using Wright stain and Giordano Buffer. A staining time of three minutes followed by 10 minutes with the buffer has proved satisfactory

for characterizing the leucocytes. Leucocytes are studied under oil immersion on both cover-slipped and uncover-slipped slides.

The acid hematin method of determining hemoglobin content of the erythrocytes was used. The drawback of this method for pocket mouse work is the requirement for 0.02 ml of blood. However, since the concentration transmittance curve is linear over a wide range of concentrations, the sample dilution can be taken at twice that of the standard so that only 0.01 ml of blood is required. Even with this modification only six animals gave sufficient blood to obtain hemoglobin readings.

The phase microscope method of direct platelet counting was used.

RESULTS

Table 1 presents the results of total red cell counts, white cell counts, hematocrit determinations, and differential counts on both male and female adult Perognathus longimembris tail blood.

Mice were sampled during a nine month period extending from July 1962 through April 1963. Except where noted, animals were bled only once.

Mean erythrocyte counts ranged from 11.9 X 10^6 cells/min^3 to 15.5 X 10^6 cells/mm^2. Mean leucocyte counts were considerably more variable ranging from 6.6 X 10^3 to 32.4 X 10^3 cells/min^3.

The high mean value (32.4 $\pm$ 24.0) on 13 December 1962 was the second bleeding for these animals and there is a suggestion of infection due to the prior tail cut. The high standard deviation for the leucocyte value on 20 December 1962 which was the third bleeding for these animals indicates a continued infection at least in some of the animals.

Hematocrits of Perognathus tail blood are high. Mean hematocrit values range from 52-59%. A sample low of 46% and a high of 62% was noted; however, for the most part sample values clustered about the mean.

The mean corpuscular volume is calculated from the hematocrit and erythrocyte values using the following formula:

$$\text{Volume Index} = \frac{\text{Hematocrit x 10}}{\text{RBC in million per mm}^3} \text{ cubic microns}$$

An average MCV of 41.5 cubic microns was obtained using this calculation. This value conforms with those given for other mammalian erythrocytes having approximately the same diameter as that of the pocket mouse.

	DATE	RBC* $X10^{-6}$ $\bar{X}$	S.D.	WBC $X10^{-3}$ $\bar{X}$	S.D	HCRT % $\bar{X}$	% LYMPHOCYTES $\bar{X}$	
FEMALES	7/25/62	12.6 ± 1.1	(10)	18.2 ± 2.2	(5)	55 (10)	---	
	8/3/62	12.8 ± 1.1	(5)	11.5 ± 3.9	(5)	52 (5)	---	Repeats of 7/25/62 mice
	8/6/62	13.4 ± 0.8	(5)	13.3 ± 0.8	(5)	55 (4)	---	
	8/20/62	13.1 ± 0.9	(10)	15.4 ± 3.4	(10)	55 (10)	---	
	8/29/62	11.9 ± 0.5	(7)	13.7 ± 3.8	(7)	56 (6)	---	
	9/17/62	14.4 ± 0.7	(5)	10.8 ± 1.6	(5)	52 (5)	83.4 (5)	
	9/28/62	12.3 ± 1.3	(5)	18.7 ± 2.8	(5)	---	---	Repeat of 9/17/62 mice
	11/1/62	12.7 ± 1.9	(9)	13.8 ± 4.1	(9)	56 (10)	---	
MALES	11/21/62	13.6 ± 1.7	(5)	7.5 ± 4.7	(5)	52 (5)	76.0 (5)	
	11/23/62	14.2 ± 0.7	(5)	8.8 ± 2.2	(5)	53 (5)	74.2 (5)	
	11/25/62	13.8 ± 0.9	(5)	11.4 ± 2.3	(5)	53 (5)	82.8 (5)	
	11/27/62	14.3 ± 1.5	(5)	9.1 ± 3.0	(5)	56 (5)	68.6 (5)	
	11/29/62	13.6 ± 1.6	(5)	10.3 ± 3.2	(5)	56 (5)	78.4 (5)	
	12/7/62	12.6 ± 1.2	(10)	11.2 ± 3.2	(10)	52 (6)	---	
	12/13/62	12.7 ± 1.3	(9)	32.4 ± 24.0	(9)	57 (10)	---	Repeats of 11/21/62 to 11/29/62 mice
	12/20/62	12.3 ± 0.9	(10)	16.2 ± 13.4	(10)	52 (3)	---	
	12/21/62	15.2 ± 1.1	(5)	17.8 ± 7.1	(5)	59 (5)	---	
FEMALES	3/27/63	13.4 ± 1.8	(5)	11.4 ± 5.9	(5)	53 (5)	82.9 (5)	
	3/29/63	13.4 ± 1.8	(5)	8.8 ± 6.5	(5)	53 (5)	84.9 (5)	
	3/31/63	12.0 ± 1.0	(5)	7.0 ± 1.7	(5)	53 (5)	86.6 (5)	
	4/2/63	15.5 ± 2.2	(5)	6.9 ± 2.6	(5)	56 (4)	73.1 (5)	
	4/4/63	13.1 ± 0.5		6.6 ± 3.7	(5)	55 (4)	69.2 (5)	

Table 1. Peripheral Blood Values of Adult Perognathus longimembris Sampled Periodically From Holding Facility. Figures in Parentheses Indicate Number of Animals in Group.

* RBC and WBC / mm^3

Erythrocytes and leucocytes were measured with an ocular micrometer under oil immersion. Measurements were made on cells in the thin, rainbow-colored section of the smear where the film is evenly spread and thin enough so that the cells be flat and abutt but do not overlap. The results of these measurements are presented in Table 2.

Observations of blood smears indicate there are no major morphological differences between pocket mouse blood cells and those of other mammals.

Lymphocyte values ranged from 45-94% over the course of this study. Mean values ranged between 68.6 and 84.9%. Neutrophils and lymphocytes together compose greater than 99.5% of the total leucocytes in peripheral blood. Monocytes compose less than 0.5% and basophils less than 0.1% of the total leucocyte count. Eosinophils were never found in peripheral blood smears.

Hemoglobin content of P. longimembris blood has a mean value ranging from 15.5 to 17.9 grams per 100 ml (Table 3). Individuals had hemoglobin value as low as 12.8 gm % and as high as 18.9 gm %. The high hemoglobin values obtained are consistent with the high hematocrit of the animal.

Platelets were counted on just one occasion during this study. The mean value of 4 mice was 834,000 cells per mm^3.

Table 4 presents data on a limited number of adult P. formosus of both sexes. Comparison with tables 1 and 3 suggests that blood values of the two species are very similar.

In table 5, RBC counts and WBC counts are presented on a series of animals (P. longimembris) which were bled from the tail on one day and from the heart two days later. It was noted that leucocyte counts made on heart blood are significantly lower than those made on tail blood.

DISCUSSION

Until very recently blood values were available for only a few species of small wild mammals. This paucity of data reflects in part technical difficulties involved in obtaining blood samples from extremely small forms. This is especially true when it is not desirable to sacrifice the animal.

No doubt a strong requirement for baseline blood values in feral population had to arise before investigators were motivated to overcome these difficulties. Earlier, this motivation arose from a desire for knowledge of blood dynamics in hibernating small mammals (4). More recently the need for blood values of rodent populations in

Kind	Number Samples		Diameter (Microns)		
	Animals	Cells	Average	Max	Min
A. Erythrocytes	6	120	6.8	8.2	4.5
B. Lymphocytes					
Group I Small	3	30	8.2	9.6	6.2
Group II Large	3	30	10.2	11.2	8.3
Group I & II	3	60	9.2	11.2	6.2
C. Monocytes	3	22	12.9	15.0	10.4
D. Granular Leucocytes					
Neutrophils	3	30	12.0	14.2	10.0
Basophils	3	3	10.4	11.7	9.2

Table 2. Size of Formed Elements in Perognathus longimembris Blood as Determined by Ocular Micrometer.

HEMOGLOBIN	GM%		
DATE	$\bar{X}$	Range	No. Mice
8/3-8/29/62	15.5	(12.8-17.4)	6
3/27/63	15.5	(14.9-16.8	5
4/4/63	17.9	(16.5-18.9	5

PLATELETS	Cells/mm^3		
DATE	$\bar{X}$	Range	No. Mice
4/4/63	834 X 10^3	(59.8 X 10^3- 99.3 X 10^3)	4

Table 3. Hemoglobin and Platelet Values of Female Adult Perognathus longimembris

DATE	RBC X $10^{-6}/mm^3$		WBC X $10^{-3}/mm^3$		HCRT %		HEMOGLOBIN GM%	
	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	Range	$\bar{X}$	Range
2/7/62					57.5	(49-65) (4)		
2/12/62					52.5	(44-60) (9)	16.6	(15.5-18.4) (10)
2/15/62	12.1 ± 1.0 (9)*		9.23 ± 5.5 (9)		52.3	(48-56) (8)	17.1	(14.5-20.7) (9)

Table 4. Peripheral Blood Values of Adult Perognathus formosus of Both Sexes.
* Number of Animals

DATE	RBC X $10^{-6}/mm^3$		WBC X $10^{-3}/mm^3$	
	$\bar{X}$	S.D.	$\bar{X}$	S.D.
4/17/62 (Tail)	13.6 ± 0.8 (11)*		12.1 ± 5.6 (11)	
4/19/62 (Heart)	10.2 ± 2.0 (11)		3.9 ± 2.3 (11)	

Table 5. Heart Blood vs Tail Blood of Adult Perognathus longimembris
* Number of Animals

radiation contaminated areas has added impetus to such studies.

The list of small mammals for which various blood values have been reported include: the least shrew, Cryptotis parva (2); the little brown bat, Myotis lucifugus (2); the deer mouse, Peromyscus spp. (2, 7); the harvest mouse, Reithrodontomys spp. (2, 7); and the pine mouse, Microtus pinetorum (2). Larger forms of wild rodents which have been investigated are: the cotton rat, Sigmodon hispidus (2); the rice rat, Oryzomys palustris (2); the muskrat, Ondatra zibethicus (2); and the kangaroo rat, Dipodomys merriami (3).

Comparisons of values obtained for Perognathus blood with these published values indicates that Perognathus blood is not remarkably different.

High erythrocyte counts and hematocrits are typical of very small mammals. The genera Perognathus, Peromyscus, Reithrodontomys, and Microtus all have erythrocyte counts greater than 10 million per cu mm. The mean erythrocyte volume of these forms ranges between 39.5 and 42.5 cubic microns.

Erythrocyte counts reported for Cricetidae show an interesting inverse relationship with body weight (2). A similar increase in erythrocyte number with decreasing body weight appears in the three species of Heteromyidae for which RBC counts are available. Perognathus longimembris weighing approximately 8.5 grams has a mean RBC count of 13.2×10^6 cells per mm^3. This is slightly greater than that of the harvest mouse, which incidentally has a mean body weight of 8.4 grams. P. formosus weighing approximately 20.0 grams has a RBC count of 12.1×10^6 cells per mm^3, and Dipodomys merriami weighing 30 to 48 gms have a mean RBC count of 8.5×10^6 cells per mm^3.

Before any significance can be attached to any such correlation, additional Heteromyids will have to be examined. Differences between deep blood and peripheral blood must also be considered. Observations in this laboratory suggest that significant differences occur between heart and tail blood of Perognathus. This is also known in the laboratory mouse (6). Some of these differences are seen in table 4 which compares red cell and white cell counts of heart and tail blood of the same animals. In addition, Perognathus tail blood appears to have a more rapid clotting time and is much more viscous than heart blood.

Normally hematocrit values are directly correlated with total erythrocyte counts. Unless erythrocytes are abnormally large or small or there are plasma changes, hematocrits indicate the concentration of erythrocytes. Hematocrits in Cricetidae range from 42.8 to 51.0%. Values reported for Peromyscus range from 44.4 to 52.2%.

The value given for Dipodomys is approximately 47.0%. Compared to all of these Perognathus hematocrits, ranging from 52 to 59%, are high. Similarly, hemoglobin values reflect erythrocyte concentration.

Hemoglobin values ranging from 14.3 to 17.1 grams percent for deer mice are comparable to those for Perognathus. The value given for Dipodomys, 13.1 grams percent, is slightly lower than that of Perognathus.

Hemoglobin concentration and hematocrit ratios showed significant seasonal variations in both confined and free-living deer mice (7). Since the Perognathus used in this study were maintained at constant temperature, no seasonal variation was expected nor was it found.

Leucocyte counts are extremely variable even within species of wild populations (2). Perognathus leucocyte values show large standard deviations. Part of this variation may be due to the blood collecting technique but there are indications that something more than artifact is operating. More work on the effect of diurnal rhythms on leucocyte numbers is suggested. Except for the muskrat, leucocyte values given for the Cricetidae and that given for Dipodomys are lower than that of Perognathus.

Lymphocytes compose 60-90% of the total leucocyte numbers in most rodents (5, 6). The ratio of lymphocytes in Perognathus falls within this range suggesting again that Perognathus blood is not strikingly different from other rodents. This fact is corroborated by the observation that morphologically Perognathus blood cells are typically mammalian.

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KARYOTYPE ANALYSIS OF PEROGNATHUS SPECIES

D. F. Mitchell and J. W. Towner

INTRODUCTION

The genus Perognathus (family Heteromyidae) consists of some 26 described species endemic to the western and central United States and Mexico, and extending into southwestern Canada. Even though the various species are quite divergent, structural intermediates lead to the inclusion of all forms into a single generic group. These pocket mice are highly specialized for arid and semi-arid environments. They are burrowing animals with diurnal and seasonal behavior patterns adapted to the extremes of their habitat. Food consists mostly of seeds, though some species may supplement this diet with some green vegetation and animal matter. Probably no species drink any significant amount of water, and none seem to require free water during their life span. These needs are met by water contained in dry seeds and by metabolic water. The life span for most species probably averages one season, however, they can be maintained for five or six years in captivity.

The ecological adaptations are also descriptive of the other genera which are included in the Heteromyids, the Kangaroo Rat (Dipodomys), Kangaroo Mice (Microdipodops, and Spiny Pocket Mice (Liomys and Heteromys). Perognathus,however, comprises a distinct morphological group which includes some of the smallest rodents in North America. The genus is divided into two distinct subgenera, Perognathus and Chaetodipus. The former contains 12 species in four species groups, the latter 14 species in six species groups.

The highly specialized nature of this genus, coupled with the morphological divergence of the various species and subspecies, makes it particularly interesting from the viewpoint of evolutionary relationships. A prior description of the karyotypes of two species, P. longimembris and P. formosus, indicated that the morphological divergence might be reflected in variation in the chromosome constitution. The description of five additional species verifies this diversity and suggest the value of still further cytotaxonomic study of this genus.

MATERIALS AND METHODS

The mice for the present analyses were taken from native populations in southern Nevada, Arizona and California and maintained in the laboratory for 3 to 18 months. The animals were sacrificed by suffocation and chromosome analyses were made from bone marrow, kidney, spleen, heart, and lung tissue. The chromosome preparations of bone marrow cells were made without prior *in vitro* culture or *in vivo* colchicine administration (3).

Tissues of the organs were dispersed with 0.25% trypsin and grown in milk dilution bottles in enriched Eagles MEM supplemented with 20% agamma horse serum, 5% newborn agamma calf serum and 2% beef embryo extract. After 5 to 8 days growth in an incubator ($37^{o}C$, 5% CO_2), the cells were treated with colchicine (0.8 gamma per ml, 2 hours), and resuspended with trypsin. The cell suspension was spun at 1000 rpm for 5 minutes and the pellet resuspended in 0.8% sodium citrate for 30 minutes. An equal volume of cold acetic-alcohol fix (1:3) was gradually added to the hypotonic saline and allowed to stand for 30 minutes. Following 2 changes in cold 3:1 fix, the cell suspension was applied to slides, air dried, and stained in aceto-orcein. The karyotype of *P. baileyi* in figure 4 was from a lung cell while those of *P. formosus* (Figure 3) and *P. penicillatus* (Figure 5) are from marrow. The remaining figures were from single cells of kidney. All photographs were made with phase contrast optics and are reproduced at 3200 X in figures 1-5.

DESCRIPTION OF KARYOTYPES

The taxonomic relationships of the seven species which have been analyzed are indicated in Table 1. (It should be noted that corrections to previously reported chromosome counts are contained in this description of *P. formosus* and *P. longimembris*. Improved techniques have resulted in the identification of three additional pairs of acrocentric microchromosomes in *P. longimembris*, and one additional small metacentric pair and a minute acrocentric pair in *P. formosus*.) The karyotype variation appears to be consistent with the taxonomy. *Perognathus flavus* has a diploid chromosome number of 50 (Figure 1), with a series of 20 pairs of sub-median to median metacentrics which gradually decrease in length. Thus far it has not been possible to break this series up into smaller groups, except for distinguishing the smallest pair in which the centromere is median. The five pairs of acrocentric chromosomes can be separated into two large pairs, two intermediate pairs, and one pair of dots. The X and Y chromosomes have not been identified.

Perognathus amplus, with a diploid number of 56, also has a series of 20 pairs of submedian to median metacentrics including a small distinguishable pair (Figure 2). Four of the smaller metacentrics can be identified by the presence of satellites. There are 8 pairs of acrocentrics which can be separated into 2 large pairs, 4 intermediate pairs, and 2 small pairs. The X and Y chromosomes have not been identified.

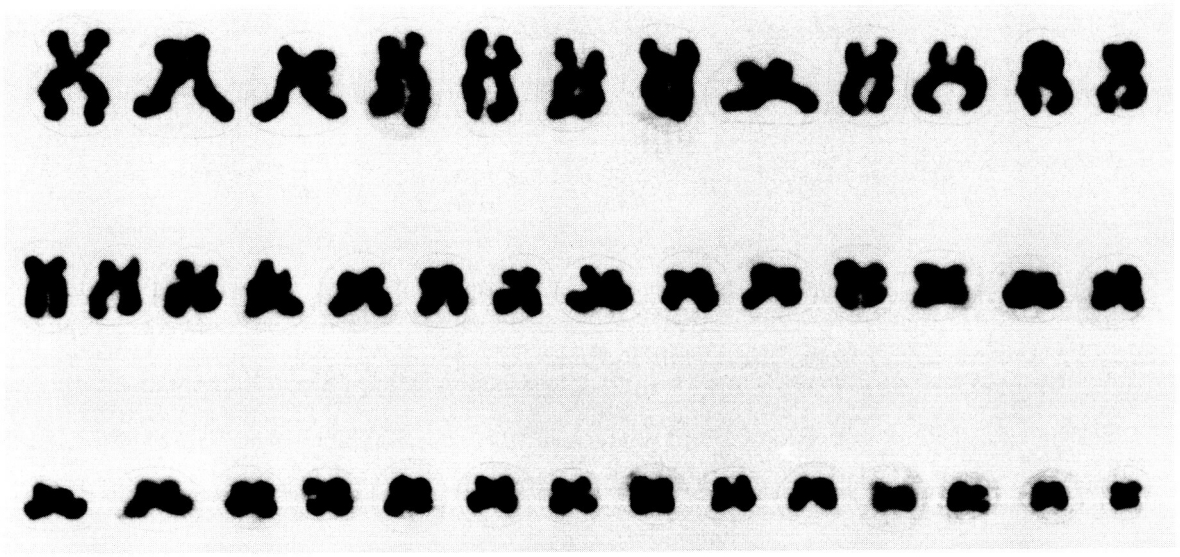
Perognathus longimembris also has a long continuous series of metacentrics, with 16 rather than 20 pairs (Figure 2). The X chromosome is contained in this series, but is not distinguishable. The 13 pairs of acrocentrics include 4 long pairs and a decreasing series of 9 pairs. The small Y chromosome is identifiable in this case, at least in the original material.

While the three species described above, all members of the subgenus Perognathus, have very similar karyotypes, the other representative of this group, P. formosus, is quite distinct (Figure 3). There are a total of only 18 pairs of chromosomes, with 8 pairs of large submedian metacentrics, a smaller submedian X chromosome, and a very small metacentric pair. The small acrocentrics include a series of 7 pairs of gradually decreasing length, and a minute pair of dot chromosomes, The Y chromosome is also a small dot.

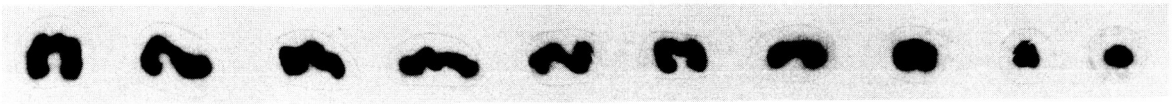
The remaining three species are members of the subgenus Chaetodipus. P. baileyi (Figure 4) with a total of 23 pairs, has 6 median metacentrics of gradually decreasing length, a submedian metacentric of smaller size, one pair of small median metacentrics, a series of 11 pairs of small acrocentrics, and a distinct very small acrocentric. Some of these chromosomes classified as acrocentrics may have very short arms distal to the centromeres and therefore may actually be metacentrics. The sex chromosomes have not been identified in this species.

Our study of P. fallax confirms the number 2N = 44 previously reported by Cross (1). This species has 6 pairs of median metacentrics, one pair of smaller submedian metacentrics, a small metacentric, and a series of 14 pairs of acrocentrics ranging from a relatively large pair to a pair of dots, (Figure 4). These "acrocentrics" are similar to those found in P. baileyi described above. The X and Y chromosomes have not been identified.

Perognathus penicillatus is distinct from the other species in that there are only 3 metacentrics, each identifiable. The first pair is a long submedian, the second a long median and the X is a shorter submedian metacentric. The remainder of the karyotype consists of a series of 20 pairs of acrocentrics, the longest of which exceed the X chromosome in length, but ranging continuously down to a pair of dot chromosomes. The Y chromosome is a small acrocentric. There is a suggestion that some of these acrocentrics may also have very short arms.

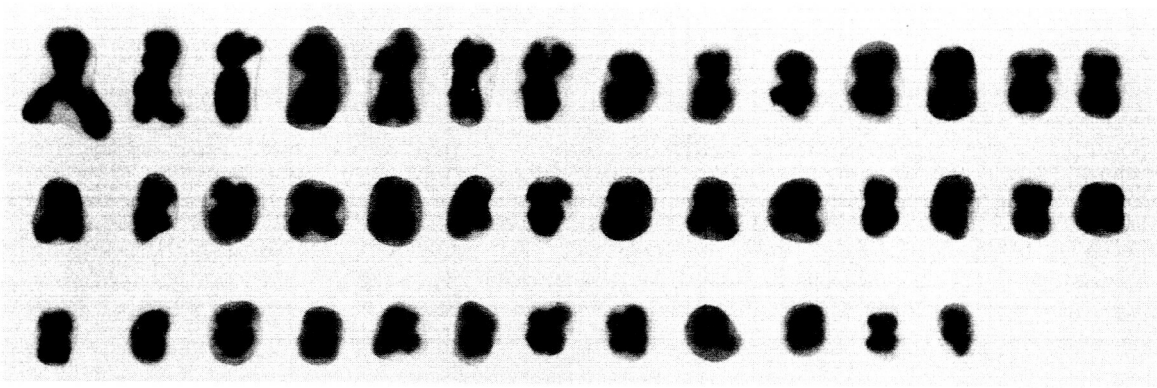


40 Metacentric

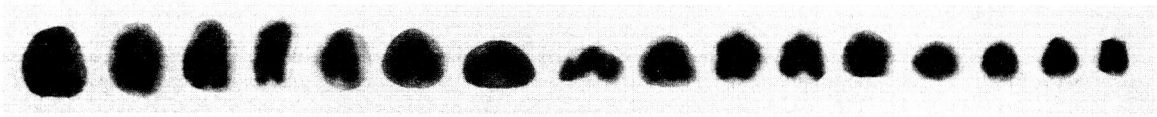


10 Acrocentric

Figure 1. Karyotype of P. flavus female, X3200



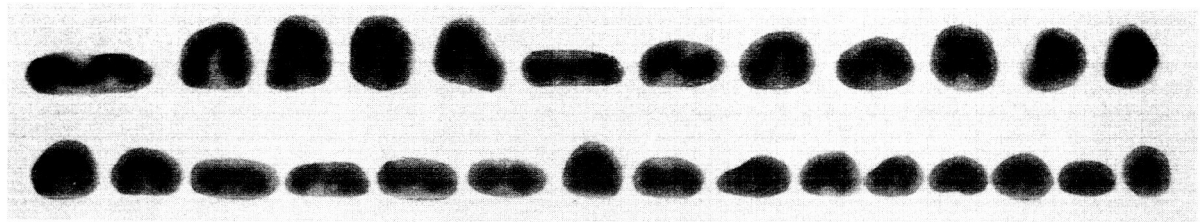
40 Metacentric



16 Acrocentric

P. amplus, female

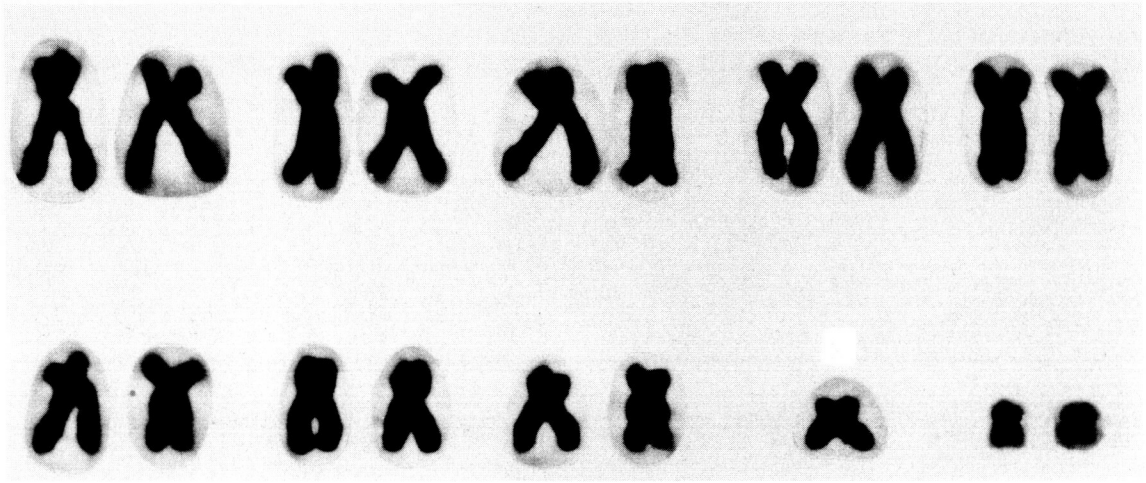
30 Metacentric, X



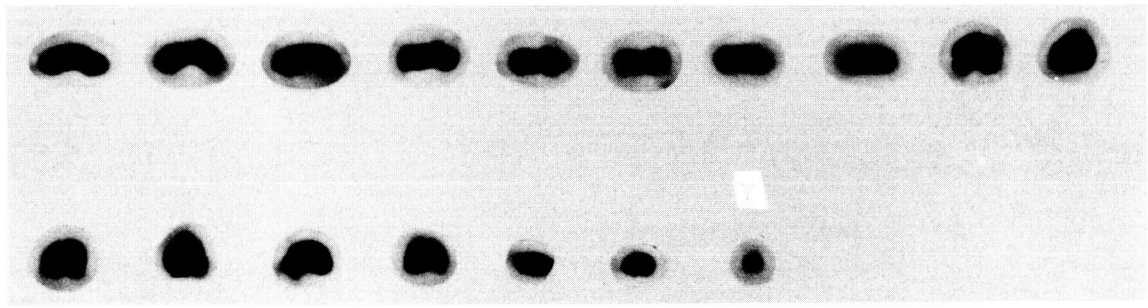
26 Acrocentric, Y

P. longimembris, male

Figure 2. Karyotypes of P. amplus female and P. longimembris male, X3200

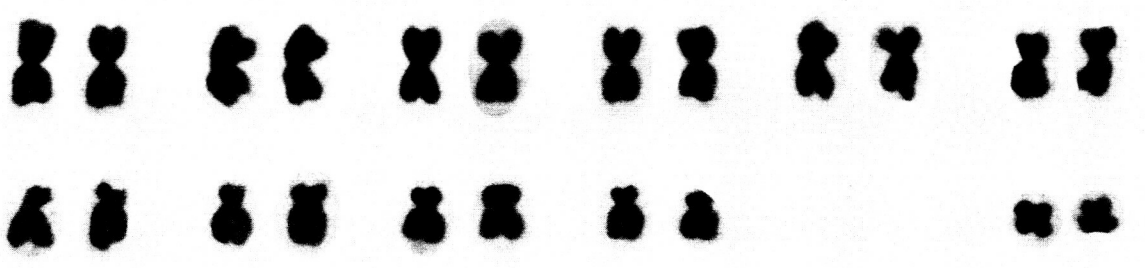


18 Metacentric, X

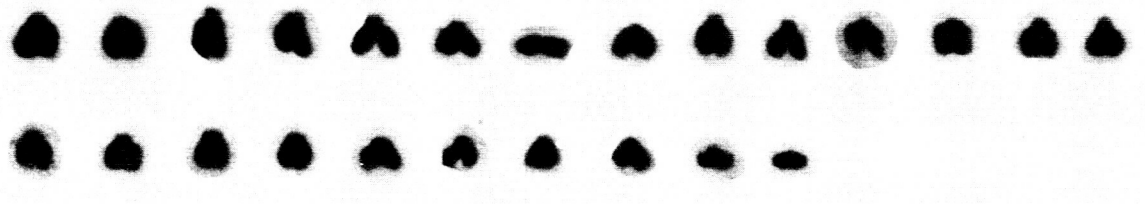


16 Acrocentric, Y

Figure 3. Karyotype of P. formosus male, X3200

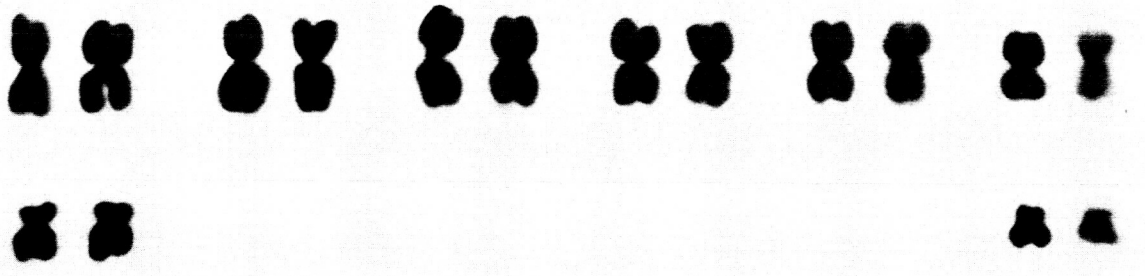


22 Metacentric

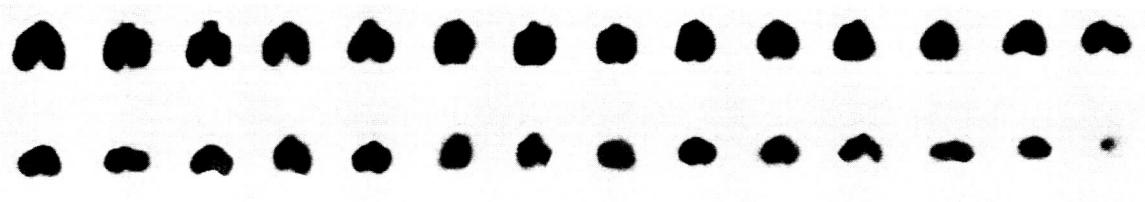


24 Acrocentric

P. baileyi, female



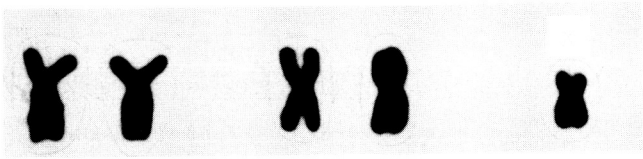
16 Metacentric



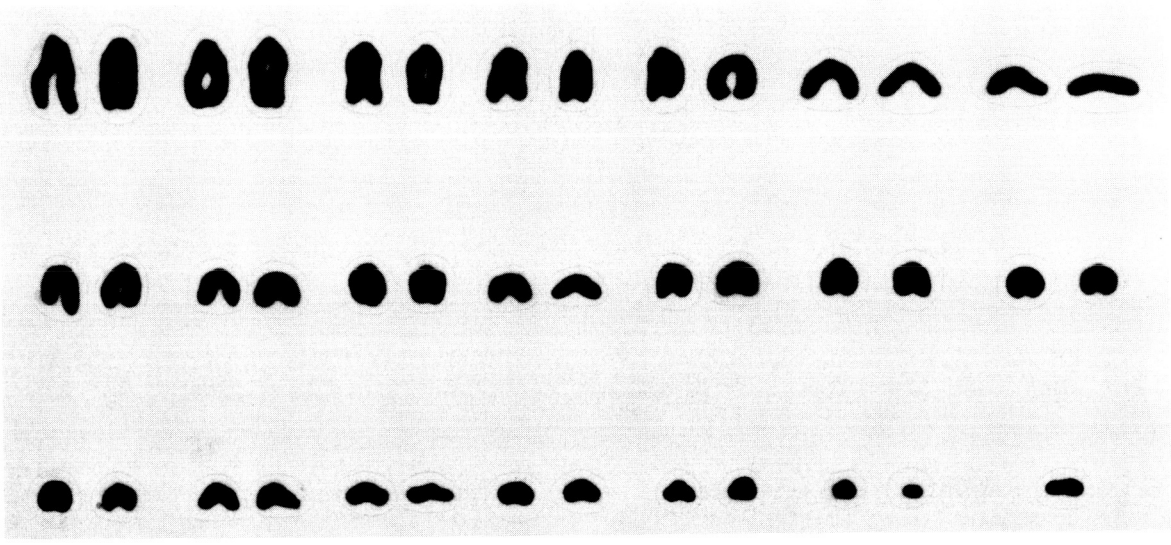
28 Acrocentric

P. fallax, female

Figure 4. Karyotypes of P. baileyi female and P. fallax female, X3200



4 Metacentric, X



40 Acrocentric, Y

Figure 5. Karyotype of P. penicillatus male, X3200

DISCUSSION

Two of the species included in this analysis are included in the same species-group according to accepted taxonomy (2), based on morphological characters (Table 1). These species, Perognathus amplus and P. longimembris might, therefore, be expected to have quite similar karyotypes. The total chromosome numbers are the highest found in this sampling of the genus, with P. longimembris having one pair (2N = 58) more than P. amplus (2N = 56). A difference also exists in the relative proportion of metacentrics and acrocentrics. P. longimembris has four less metacentrics and five more acrocentrics than P. amplus. This suggests the possibility of centric fusion in the genetic divergence of these two species. However, other processes would be required to explain the different ratios of metacentrics to acrocentrics, as well as the differences in total numbers of chromosome arms.

The karyotype of Perognathus flavus is quite similar to that of P. amplus. The long series of metacentrics appear to be almost identical. P. flavus, however, has three fewer acrocentrics than does P. amplus. A suggestion of centric fusion exists again in the comparison of P. flavus and P. longimembris (Table 1). If eight of the acrocentric chromosomes of P. longimembris are combined to form four metacentrics the proportion would be identical to that of P. flavus. This may, of course, be happenstance but it strongly suggests that centric fusion may have been involved in the karyotypic evolution of these species. On the basis of these comparisons alone, it might also be suggested that these two species are more closely related than is apparent from their taxonomic placement.

The karyotype similarities between these three species is striking. The metacentric series are almost identical, except for the reduction in number in P. longimembris. In the largest and smallest metacentrics, where comparisons are possible, the chromosomes are certainly very similar. The same is true of the four largest pairs and the smallest pair of acrocentrics. The close relationship of these species is verified by these karyotype descriptions.

The fourth representative of the subgenus Perognathus, P. formosus, is quite divergent. It has only 18 pairs of chromosomes. Eight pairs of the metacentrics are unusually large for rodents, much larger than in the other three species which have been described. In this case the longest chromosome can be distinguished in most preparations, and two of this series can often be identified by the more median position of the centromere. The X chromosome is distinct, and the small metacentric present in the other species is also present. The small acrocentrics appear similar to the other species in the subgenus. This karyotype is very different than those previously

Table 1. Comparative Karyotype Analysis of Some Species of Perognathus

Genus Perognathus	2N	Meta.	Acro.	M/A	Arms	Arms/ centromere
Subgenus Perognathus						
Fasciatus-group						
1. P. flavus	50	20	5	4:1	45	1.80
Longimembris-group						
2. P. amplus	56	20	8	2.5:1	48	1.71
3. P. longimembris	58	16	13	1.23:1	45	1.53
Formosus-group						
4. P. formosus	36	10	8	1.25:1	28	1.56
Subgenus Chaetodipus						
Baileyi-group						
5. P. baileyi	46	11	12	0.915:1	34	1.48
Intermedius-group						
6. P. fallax	44	8	14	0.571:1	30	1.36
Penicillatus-group						
7. P. penicillatus	46	3	20	0.15:1	26	1.13

described, suggesting that a more distant relationship may exist than that implied in the taxonomy.

Two of the species belonging to the subgenus Chaetodipus, P. baileyi and P. fallax, are also quite similar, though the ratio of metacentrics to acrocentrics differs (Table 1). Both karyotypes have the small metacentric which is present in P. formosus, and perhaps in the other species. The acrocentrics are quite comparable, except for total number. In total aspect they appear to be closely related. It also appears that the karyotype of P. formosus may be more similar to these two species than to those previously described, to which it is presumably more closely related.

P. penicillatus is the most divergent of the species; so much so that no meaningful comparisons are possible. The X chromosome appears to be similar in this species and P. formosus and the acrocentric series has some elements similar to those in P. baileyi and P. fallax. An analysis of closely related forms would be of interest.

This sampling of the karyotypes of the pocket mice has demonstrated a high degree of variation within the genus, which is consistent with the morphology. The seven species studied were selected solely on the basis of their availability, without any prior consideration of taxonomic relationships. A number of species groups are not represented. The wide divergence of the karyotypes of some species does not permit a serious consideration of karyotype evolution in the genus without additional descriptions. It would be of interest to obtain a larger series of species, thereby permitting a more detailed analysis. In view of the highly specialized nature of the genus, and the distribution patterns of the various species and species groups, a complete cytotaxonomic study would be of value.

SUMMARY

1. The karyotypes of seven species of pocket mice have been described. The diploid chromosome number ranges from 36 to 58 in the various species.
2. The ratios of metacentric to acrocentric chromosomes and the chromosome number and morphology are highly variable. Karyotypic divergence is, however, generally consistent with the taxonomy based on morphological characters.

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BREEDING OF HETEROMYID RODENTS

T. Tagami

Little is known of the reproductive behavior of pocket mice. Gravid females trapped in the field have been reported to be carrying four to six embryos and litters of four have been born in the laboratory by field bred animals. Population studies in the field suggest that only one litter a year is produced.

In an attempt to establish a breeding colony of P. longimembris and P. formosus, three categories of experiments were undertaken: (1) pairing experiments in which males and females were either kept together in large one by two-foot terraria for long periods, or individuals were allowed to mix intermittently; (2) external examination of females and analysis of vaginal smears in an attempt to establish a normal estrus cycle; and (3) attempts to stimulate reproductive behavior by hormones either in the food or injected subdermally. In the course of these studies only one copulation was observed and no litters were produced.

Despite the lack of success in the above studies, Perognathus californicus, P. penicillatus, and P. flavus have been bred successfully in captivity (Eisenberg and Issacs). The laboratory data show that the average litter size for these species is four, that these species reach adult proportion in three months, and that the gestation period is approximately 26 days.

It is apparent that the use of pocket mice as standard laboratory material may be limited because of the difficulty of establishing a laboratory breeding colony. The general usefulness of this group, however, as a research tool warrants continuing efforts to solve the breeding problem.

ANNOTATED LITERATURE REVIEW ON THE LABORATORY BREEDING OF HETEROMYID RODENTS

1. Chew, R. M., Reproduction of Dipodomys merriami merriami in Captivity. J. of Mamm. 39: 597, 1958.

A. Method: Animals bred in 24 x 18 x 12 inch cages with two compartments. Animals housed in 10 x 16 x 11 inch outdoor cages in which desert conditions were simulated. Brown glass bottles were provided for nesting.

B. Typical changes during female estrus:

1. Swelling and protrusion of the vulvar region with bloody mucous discharge.
2. Wide opening of vulva.
3. Rapid regression and tight closure. The open condition lasted an average of 6 days (3 day minimum and 11 day maximum). The cycles occured on the average of every 25 days (13 days minimum and 45 days maximum).

C. Conclusions:

1. Success depends on selection of females tolerant to captivity and able to maintain a body weight normal to wild animals. Underweight females did not undergo estrus.
2. Daily fluctuations in temperatures as obtained by outdoor cages may be necessary if indoor temperatures are too stable.

2. Laboratory of Nuclear Medicine and Radiation Biology, UCLA, Unpublished Data. May 1960. Three Breeding Trials of Dipodomys merriami resulting in two pregnancies and one litter.

A. Method: Animals housed in 8 x 12 x 8 inch terraria. Females were observed until the onset of estrus. Females were then placed with a male with descended testes in 12 x 24 x 12 inch cages for 24 hours.

B. Temperature was maintained at 10°F below outdoor temperature. The maximum indoor temperature was 90°F during mid-afternoons and a minimum in the low 70's during the night.

3. Butterworth, B. B., The Breeding of Dipodomys deserti in the Laboratory. J. of Mamm. 42: 413, 1961.

A. Method: Male and female animal caged in a 6 x 8 meter pen with nesting chambers in opposite corners. Temperature fluctuated between 60 and 70°F daily. Light provided through two northern exposures.

B. Results: 7 litters or a total of 23 animals.

C. Conclusions: A large cage is necessary so the male can escape the female's fierce aggressiveness during diestrus.

4. Butterworth, B. B., A Comparative Study of the Growth and Development of the Kangaroo Rat Dipodomys deserti and D. merriami. Growth 25: 127, 1961.

A. Method: Paired animals kept in 3 x 4 meter cages with several inches of

desert sand covering the floor. Nesting sites consisting of brown glass bottles, empty cardboard mailing tubes and cardboard boxes were provided.

B. Results: 8 litters of D. deserti and 4 litters of D. merriami.

5. Eisenberg, J. F., and Isaac, D. E., The Reproduction of Heteromyid Rodents in Captivity. J. of Mamm. 44: 61, 1963.

A. Method: Dipodomys nitratoides, D. merriami and Liomys pictus were paired in 5 x 7 x 2 foot cages. These three species, D. panamintinus and Perognathus flavus were also bred in 74 x 15 inch cages but with a center portion separating the animals. Successful breeding of Perognathus californicus, D. nitratoides and D. panamintinus also done by simultaneous introduction of male and female into an arena with a floor space of 6 sq. ft. followed by an encounter period of 30 to 60 minutes.

B. Conclusions: Liomys pictus, Dipodomys nitratoides and D. panamintinus kept in pairs continuously resulted in no litters. It appears that continuous proximity of the male may cause the female to stop cycling and enter a prolonged anestrus. Successful breeding demands that the sexes dwell separatly, coming together for mating with only a brief pairing interval.

Northrop Space Labs. NASA-91

UNPUBLISHED PRELIMINARY DATA

APPLICATION TO SPACE BIOLOGY RESEARCH

R. G. Lindberg and D. F. Mitchell

The information presently available concerning the anatomy, histology, and physiology of pocket mice indicates that they are good experimental mammals, admirably adaptable to biopack experimentation by virtue of their specialized adaptations to desert conditions. These adaptations simplify experimentation in balloon or orbital vehicles by minimizing life support requirements. Ease of maintenance coupled with their small size permits design of a biopack containing as many as 75 individual subjects per pound of animal payload. This permits a greater flexibility in the design of experiments, and a greater return of information from successfully completed experimental flights. The requirements for statistically significant numbers of experimental subjects cannot be overstated. Biological responses are innately variable, and the data from experiments must be amenable to statistical analysis. The result must be a valid statement of the reliability of the analysis and interpretation of the experimentally-obtained data. The value of Perognathus is therefore not necessarily in the study of temperature regulation, water metabolism, hibernation, or radiation resistance, but rather in the exploitation of these specialized adaptations in specific experiments concerned with the survival of a mammalian system under the stresses imposed by the space environment.

On the basis of experimental data obtained in the course of this study, it is suggested that Perognathus may have a useful role in each of the following experimental categories.

Radiobiology

Ionizing radiation in space is a problem yet to be defined in terms of its effect on biological systems. Extrapolation of physical measurements of space radiation to biological effects on higher animals is tenuous. The biological responses to ionizing radiation are random in nature and are related to the kind and quality of the radiation, the rate and geometry at which the ionization is delivered, the particular tissues or even species used to measure response, and the age and sex of the individual.

These variables are further complicated in space by the presence of mixed radiation sources and energies which produce both additive and synergic responses. It is doubtful, for example, whether the effects of a given amount of ionization produced by cosmic radiations can be compared with the effects a similar amount of ionization produced by X-rays or gamma rays with lower specific ionizations.

Perognathus formosus, one of the species studied in this program, has been shown to have relatively few and large chromosomes in comparison to most rodents. This karyotype can be used readily in cytogenetic studies concerned with the effects of ionizing radiation, in fact such a study concerned with fallout effects in the Nevada Test Site is now in progress. P. formosus is remarkably resistant to radiation, with an LD_{30}^{50} of 1280 r of cobalt^{60} irradiation. The relationship between this high resistence and radiosensitivity at the chromosome level is being studied in the program referred to above. The value of cytogenetic effects as basic indices of radiation damage is well documented. The work completed in this program and that being pursued at the present time may provide an experimental mammal, with the advantages previously discussed, and with a high radioresistance. These animals might accumulate damage at the cytogenetic level but still survive to serve as a source of cells for post exposure analysis. The possibility exists of designing an experiment for exposure in a high level cosmic radiation field, containing physical dosimeters, cell cultures, and intact pocket mice. Post-flight analysis could be carried out on various tissues to determine the effects of the specific mixed field encountered.

Weightlessness

All known forms of life have evolved in a constant one g environment to which specific sensors in many organisms are known to be directly or indirectly adapted. Yet to be determined is the degree to which the total organic system is adapted. It is to be expected that extended exposure to a hypo-gravity condition will result in some malfunctioning. To some extent, and for brief exposures, compensatory and adaptive mechanisms may operate to maintain the normal state of the organism, but in time it is quite probable that major or minor pathological conditions will become manifest.

At the present time, it is difficult to meaningfully predict what the effects might be or to design definitive experiments since there are few indications of the symptoms of indices to be monitored. These must be determined on a purely theoretical basis; by constructing hypotheses as to the effects of the removal of the one g vector on the dynamics of body fluids, on the structural elements of the body (bone and musculature), and on other vital functions less obviously affected by gravity. At the present time experimentation with weightlessness will be preliminary and exploratory. It will lead, however, to more specifically defined, and more critically designed, experiments.

Perognathus has some characteristics which may be of value in zero g experiments. The primary traits are those previously described which permit large numbers of animals to be flown for extended periods under simple and constant environmental conditions. The hibernation-estivation behavior pattern of the pocket mouse involves extended periods of inactivity, and the ability to recover rapidly from torpor and resume normal activity. During the period of inactivity, weight loss is negligible. The semi-solid urine could be easily collected for later analysis. In this animal, extended periods of inactivity under confinement have no significant deleterious effect. As a consequence, the effects of weightlessness may be distinguished from those of inactivity. An exploratory exposure would be possible in which a large sample of mice would be flown for several weeks in a hypothermic state in a package carrying sufficeint shielding to minimize radiation effects. A control package in the laboratory could be exposed to simulated launch and re-entry forces, but maintained in the one g environment. Subsequent comparisons of critical functions of these two groups of animals might lead to the detection of hypogravity effects.

Non-recoverable Vehicles

All of the experiments described thus far involve the use of hypothermic animals and their subsequent retrieval. At the present time this procedure would be most likely to yield the most data per unit effort. The characteristics of the pocket mouse may also prove useful in non-retrievable remote experimentation either in close orbit or deep space vehicles. This would require instruments to detect the subject variables, and telemetering of these observations to surface stations. These requirements limit the flexibility of experimentation, but could make direct observations of immediate effects possible.

Non-recoverable vehicle experiments could make use of the behavior pattern of the pocket mouse relative to temperature and available food supply. Pocket mice could be maintained alive for extended periods in long term deep space probes by programming periodic temperature increases and the availability of food. The mice would be kept in a hypothermic state for a few weeks, the chamber warmed, the aroused mice fed, and the chamber subsequently cooled to induce a return to hibernation. As far as is known at the present time this cycle could be maintained for an indefinite period. In this way, living, healthy mice could be transported great distances in space, or they could be transported to, and maintained on, extra terrestrial bodies.

Biological Rhythms

Biological rhythms are recognized as a fundamental characteristic of living systems. Since their formal recognition by De Mairan in 1729 to the present, the question as to whether these rhythms are set by endogenous or exogenous cues or reflect

The interaction of the two has presented a formidable challenge to the experimental biologist. Recent advances in space technology now permit the placing of organisms demonstrating pronounced rhythms into earth orbits thereby providing a promising method of experimentation concerning the question of the influence of exogenous cues (Zeitgever) on biological rhythms.

PROPOSED EXPERIMENT

Experiment Title

The effect of extra-terrestrial residence on the circadian metabolic rhythm of pocket mice (Perognathus longimembris).

Working Concepts

Data from metabolic studies on pocket mice strongly suggest that Perognathus longimembris has a circadian metabolic rhythm which can be detected at both moderate (22 - 24°C) and low (10°C) environmental temperatures, at low humidities (less than 10% relative humidity) and saturated air, in the dark or under normal photoperiod, with and without food, in normal atmospheres and 100% oxygen, and in both individually housed and in grouped mice (see NSL 62-125-4). It is anticipated that placing these animals in earth orbit will elucidate the effects of exogenous factors which may influence what appears to be a dominant endogenous rhythm. While the most obvious exogenous cues to be studied are weightlessness and environmental periodicity, the experimental design is easily adaptable to provide for the input of almost any specific environmental stimuli in the isolation of space.

Experimental Design

Four units of 8 mice each (P. longimembris) to be subjected to a minimum of two weeks in orbit. At least the first week to be in a weightless condition with artificial gravitational force provided the second week if anomalies in the rhythms become apparent. Since availability of food is known to influence the depth of hypometabolism, the following feeding schedule will be maintained.

Unit one: Fed both first and second weeks.

Unit two and three: Not fed first week, fed second week.

Unit four: Fed first week, not fed second week.

Oxygen consumption of each unit to be monitored in flight with data readout adapted for computer analysis on ground. If circadian rhythm not present first week, and first unit with normal metabolic level, artificial gravity to be introduced second week in form of centrifugal force developed by spinning either vehicle or experimental package.

Potential Conclusions

(1) If units one and four maintain normal metabolic rate, with or without rhythm, during the first week, it is demonstrated that weightless animals can feed

adequately, and physiological function must be proceeding relatively normal.

(2) If there is no circadian rhythm the first week, and animals are feeding, and if rhythm is regained during second week (under artificial gravity), this is evidence that gravity is the clue for metabolic rhythm.

(3) If circadian rhythm persists in weightless condition and animals are feeding, gravity is not an important clue to setting of rhythm.

(4) If failure of feeding, and no rhythm, either under weightlessness or artificial g, then physiological functions grossly interfered with and may or may not be linked to weightless exposure.

General Experimental Requirements

1. Lead time: Weeks
2. Time:
   a. Prelaunch hold time: Not critical up to 48 hours.
   b. Orbit time: Minimum 14 days.
   c. Maximum post-flight time: Recovery unnecessary if telemetry available.
3. Radiation exposure: Not to exceed 1400 r over 14 days.
4. Environmental conditions:
   a. Temperature: Prelaunch 10° to 35°C; Flight 8 - 12°C; Recovery 10° to 35°C.
   b. Atmospheric composition: Depends upon how metabolic activity is to be monitored, e.g.,
      100% oxygen, if consumption monitored in terms of decreasing pressure;
      approx. 21%, if consumption to be monitored in terms of decreasing oxygen concentration;
      either composition of CO_2 production is measured in lieu of O_2.
      Atmospheric pressure: ~15 psi.
   c. Other:
      Animals in dark.
      Humidity to be constant, level not critical, but preferably low (<30%).
      Device for imparting spin to animal chambers or vehicle during second week to be used if desired, depending upon results of first week.
      Means controlling ammonia.
5. Operational constraints imposed by experiment:
   a. Maximum permissible acceleration: Unknown
   b. Maximum permissible spin at launch and reentry: Unknown
   c. Does slow rotation on tumbling effect experiment? Yes
   d. Other:

6. Weight estimates: Minimum of 32 mice, 9.5 grams each

Unit one: Assuming mice will remain normothermic and normally metabolic as on earth, at rate of 9 ml O_2/g hr, respiratory quotient of 0.75, caloric equivalent of oxygen of 4.8 cal/ml, food of 6 Kcal/g caloric value, evaporation at rate of 1 mg/ml O_2.

One mouse: (9.5 g) (9ml O_2/g hr) (24 hrs) (14 days) = 28.7 liters O_2 (40.2 g) used while consuming 23 grams of seed (sunflower) producing 0.75 x 28.7 - 21.5 l CO_2 or 42.1 g and losing 28.7 g water (at rate 1 mg H_2O per ml O_2).

Vehicle must provide 8 X 40.2 gm oxygen, 8 X 23 grams sunflower seed, absorbent for 8 X 42.1 gm CO_2, absorbent for 8 X 28.7 gm water.

Units two, three and four: These are to be provided with four days of food for each mouse, to be issued either at start of at beginning of second week. Animal must be provided enough oxygen to utilize this food and 50% of its own body weight. (If animals do not become torpid, and thus stay within this nutrient supply, they will have starved to death before exceeding this O_2 supply.)

To use up $\frac{(6.6 \text{ gm food})\ (6 \text{ Kcal/gm})}{4.8 \text{ Kcal/liter}}$ = 8.25 liters O_2 or 11.55 gm O_2.

To use up 4.75 gm body weight, or 1.9 gm body solids, with maximum caloric value of 9 Kcal/gm.

Units two, three and four require, for total of 24 mice

24 X (11.55 _ 5.0) gm oxygen - 397 gm oxygen

24 X 11.81 X 0.75 X 1.67 = 355 gm CO_2 absorbence

24 X 11.81 = 284 gm water absorbence

24 X 6.6 = 156 gm food as sunflower seed

LIFE SUPPORT SYSTEM FOR THE STUDY OF BIOLOGICAL RHYTHMS IN POCKET MICE - A PRELIMINARY DESIGN

W. Kuehnegger

APPROACH

The following sequential approach will govern the design and investigation of this life support system:

(1) determination of operational limitations
(2) general metabolic requirements of the specimen
(3) integration of metabolic requirements into system operation
(4) life support system schematic
(5) modular break-down
(6) the specimen module
(7) the experiment support module
(8) final description and comments.

DESIGN

Determination of Operational Limitations:

These have been taken from the existing information where possible and have been supplemented by assumptions. The total operational spectrum for this system is found tabulated below. Since the experiment consists of four different units (from prior information) the table also shows their individual limitations. (Table 1.)

General Metabolic Requirements of the Specimen:

General metabolic requirements calculated for 4 units of 8 mice each are tabulated in Table 2. The values in Table 2 are appropriately reworked yielding the Earth metabolic requirements at S.T.P. conditions per specimen day (24-hours) shown in Table 3.

Table 1. Operational Limitations

Week	Sub-System	Unit - Operation 1	2	3	4
1	Oxygen	normal	*	*	* *
	Carbon Dioxide	normal	*	*	* *
	Food	every day	none	none	4 days only
	Water Prod.	normal	*	*	* *
2	Oxygen	normal	* *	* *	*
	Carbon Dioxide	normal	* *	* *	*
	Food	every day	4 days only	4 days only	none
	Water Prod.	normal	* *	* *	*

* metabolic turnover equivalent with 50% body weight reduction
** 4 days normal plus metabolic turnover equivalent with 50% body weight reduction

Table 2. General Metabolic Requirements of the Specimen

Specimen	P. longimembris - Pocket Mouse
Number	four units of 8 mice each
Weight	9.5 grams each
Earth Metabolic Values	
Oxygen	9 ml of Oxygen/g/hr.
Carbon Dioxide	to respiratory Quotient R.Q. - .75
Caloric Equivalent	4.8 cal./ml. of Oxygen
Caloric Value of Food	6 Kcal./g
Water Production Equivalent	1 mg./ml. of Oxygen

Table 3. Earth Metabolic Requirements at S.T.P. Conditions per Specimen Day (24-Hours).

Oxygen Requirement	2.052 l. or 2.930 grams
Carbon Dioxide Production	1.540 l. or 3.045 grams
Food Requirement	Sunflower Seed 1.643 grams
Water Production	2.052 grams
Thermal Production	9.858 Kcal.

Integration of Metabolic Requirements into System Operation:

When combining the values of the operational limitations (Table 1) with the metabolic requirements per specimen day (Table 3) in conjunction with the originally specified data, this will produce the integrated metabolic values as found tabulated in Table 4.

The values of the previous Table 4 will be used to determine the individual sub-system with respect to capacity and flow rate.

The total metabolic turn-over for the complete experiment during the specified mission is shown in Table 5.

Life Support System Schematic:

The life support system schematic for the proposed pocket mouse experiment is shown in Figure 1.

Modular Break-Down:

The experiment is subdivided into:

(1) the specimen module and

(2) the experiment support module.

These are illustrated in Figure 2.

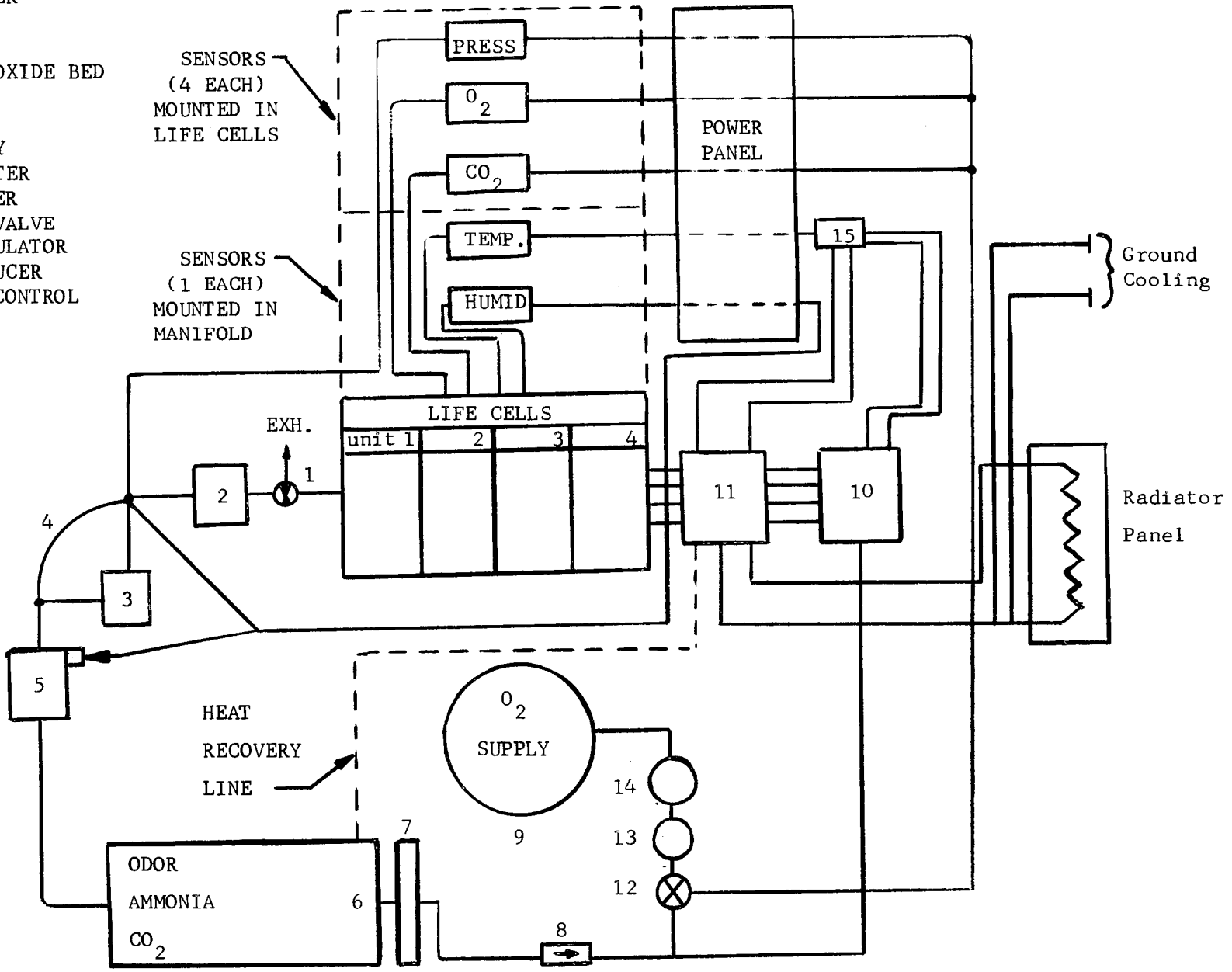
This modular concept will contribute to ease of maintenance as well as to the dynamics of the capsule.

Table 4. Integrated Metabolic Values for System Specified in Tables 1 and 3

Week	Description	Unit Weight - Grams				Sub-System Sub-Total
		1	2	3	4	
1	Oxygen Requirement	164.20	40.72	40.72	134.60	380.24
	Carbon Dioxide Production	170.50	42.30	42.30	139.70	394.80
	Food Requirement	92.00	none	none	52.58	144.58
	Water Production	115.00	28.48	28.48	94.14	266.10
	Thermal Production	552.0 Kcal	136.8 Kcal	136.8 Kcal	452.4 Kcal	1,278.0 Kcal
2	Oxygen Requirement	164.20	134.60	134.60	40.72	474.12
	Carbon Dioxide Production	170.50	139.70	139.70	42.30	492.20
	Food Requirement	92.00	52.58	52.58	none	197.16
	Water Production	115.00	94.14	94.14	28.48	331.76
	Thermal Production	552.0 Kcal	452.4 Kcal	452.4 Kcal	136.8 Kcal	1,593.6 Kcal

Note: The above values have been computed by slide rule.

1. PRESSURE RELIEF VALVE
2. DEBRIS TRAP
3. WATER ABSORBER
4. BY-PASS
5. BLOWER
6. LITHIUM HYDROXIDE BED
7. DUST FILTER
8. CHECK VALVE
9. OXYGEN SUPPLY
10. ELECTRIC HEATER
11. HEAT EXCHANGER
12. OXYGEN MET. VALVE
13. PRESSURE REGULATOR
14. PRESSURE REDUCER
15. TEMPERATURE CONTROL



LIFE SUPPORT SYSTEM SCHEMATIC

FIGURE 1

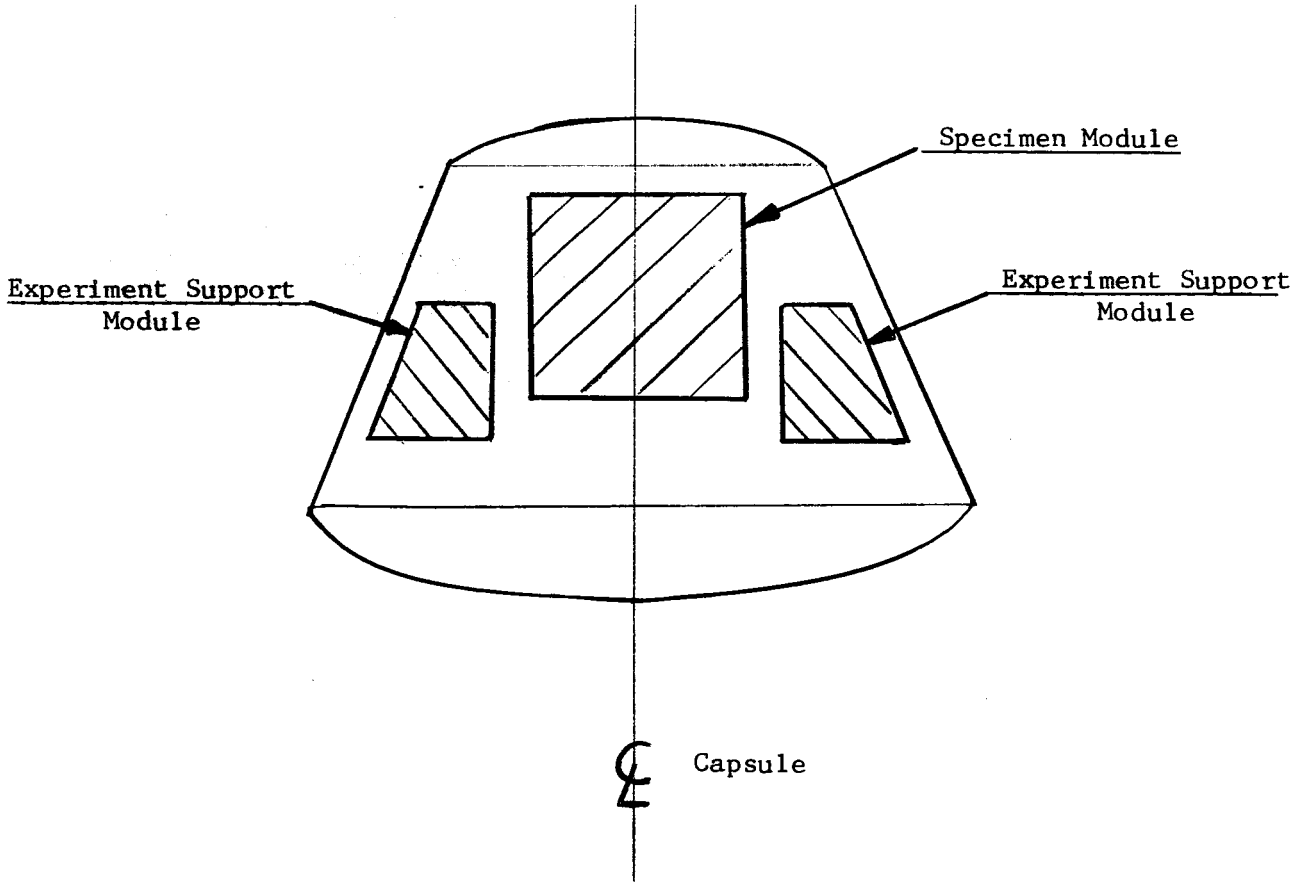


Figure 2. Module Location

Table 5. Total Metabolic Turn - Over for Complete Experiment During Specific Mission

Sub-System	Weight or Caloric Value
Oxygen Requirement	854.36 grams
Carbon Dioxide Production	887.00 grams
Food Requirement	341.74 grams
Water Production	597.86 grams
Thermal Production	2,871.6 Kcal

The Specimen Module:

The following sub-systems and components are contained within this module:

(1) the four life cells

(2) the feeder and food storage

(3) the pressure relief valve

(4) sensors and telemetry pick-up

(5) module structure

The components chosen have been computed from the governing information. They are found tabulated in Table 6.

Table 6. Components for Specimen Module

Component Description	Weight-lbs.	Volume-cub.in.	Power Requirements - watta
Life Cells	1.20		
Feeder	.68		
Food	.75		
Food Storage	.11		
Pressure relief valve	.18		
CO_2 sensors - total	.63	14.16	
CO_2 amplifiers - total	4.50	95.60	8 watts
CO_2 sensors - total	.63	14.16	
CO_2 amplifiers - total	4.50	100.20	8 watts
Module structure	2.60		
Specimen - 32	.07		
Total	15.85		

The Experiment Support Module:

This module contains the following sub-systems and components:

(1) oxygen supply

(2) carbon dioxide absorption

(3) water removal

(4) odor absorption

(5) flow control

(5) flow control

(6) thermal control

The corresponding hardware has been computed and is shown in Table 7.

Table 7. Components for Experiment Support Module

Component Description	Weight-lbs	Volume-cub.in.	Power Requirements - watts
Oxygen supply*	3.06*		
O_2 pressure vessel, 3,000 psi.	5.00	381.70	
H_2O Abs. Linde Sieve	10.60	352.00	
CO_2 Abs. LiOH bed	3.26	217.00	
Odor Abs. Act. Charcoal	.30	20.87	
Blowers - 4 used	.60	14.16	62 total
Check valve	.04	1.00	
Pressure reducing regulator	1.60		
Various connectors	.80		
Solenoid valves - 2 used	.60		14 total
Misc. hoses	.90		
Clamps	.20		
Tubing and lines	.60		
Various electrical connectors	.40		
Heater	.80		
Heat exchanger without panel	1.80		
Coolant pump	.20		28
Module structure	2.60		
Total	33.36		

* including leakage and operational venting allowance

Final Description and Comments:

Taking the values for weight from Table 6 and Table 7 and estimating the remaining volume and power requirements based on the existing figures gives final information as to the total requirements as well as their modular distribution (Table 8).

Table 8. Total Weight, Volume, and Power Requirements

Description	Specimen Module	% of Total	Experiment Support Module	% of Total	Total
Weight - lbs	15.85	32.20	33.36	67.80	49.21
Volume - cub. in.	2,030	23.20	6,720	76.80	8,750
Power Requirement	30 watts	15.00	170	85.00	200

It should be noted that nearly all tabulated values represent lengthy computations that have not been included in this preliminary investigation.

A separate description of the operational function of the life support system shown on the schematic could be added as an appendix to this report if desired at a later date. This could also include the hardware description and discuss their respective choice.

In overall this system is designed to meet the specified properties under the available and computed information.

Further it is mentioned that the above computations exclude the following:

(1) the power supply itself

(2) the space radiator panel/s

(3) the telemetry transmission

since these are assumed to be an integral part of the spacecraft itself.